# Closing the yield gap: improving production efficiency in smallholder farms of Nile tilapia through selective breeding

Samuel Bekele Mengistu

**Propositions**

1. Genotype by environment interaction studies in Nile tilapia are not worth the effort. (this thesis)

2. Log-transformed variance of deviations is the best resilience indicator to genetically improve resilience in Nile tilapia. (this thesis)

3. Patent waivers on vaccines are required to combat a pandemic.

4. Using catch data for fish stock health assessment is misleading.

5. Ethnic politics is a problem for democracy.

6. Aquaculture and chicken production are the gateway to women empowerment in developing countries.

Propositions belonging to the thesis, entitled
Closing the yield gap: improving production efficiency in smallholder farms of Nile tilapia through selective breeding.

Samuel Bekele Mengistu
Wageningen, 29 August 2022

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**Abstract**

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The aim of this thesis was to optimise the breeding program of Nile tilapia for a smallholder production system, thereby contributing to closing the yield gap. From Chapter 2 to 4, we generated information that can be used to optimize the current GIFT breeding program for Nile tilapia. Identifying the major factors contributing to the current yield gap in smallholder Nile tilapia farms is crucial and the first step to any intervention. In Chapter 2, based on meta-analysis of literature, dissolved oxygen was identified as one of the major environmental factors contributing to reduced growth and FCR. In the presence of substantial dissolved oxygen differences between the environments, genotype by environment (GxE) interaction can be expected. The output from Chapter 2 was used to design the GxE interaction experiment (Chapter 3). In Chapter 3, heritabilities for harvest weight (HW 0.18 and 0.23), thermal growth coefficient (TGC, 0.21 and 0.26), survival days (0.03 and 0.04) and size traits (0.08 to 0.45) were estimated, in an attempt to indicate that these traits can be improved by selective breeding. We estimated the genetic correlation for these traits between aerated and non-aerated ponds. The genetic correlations (rg) between the aerated and non-aerated ponds for harvest weight (0.80±0.30) and growth (0.81±0.27) indicate some GxE interactions. The less than unity genetic correlation implies that the genetic improvements gained in aerated ponds will not be fully expressed in non-aerated ponds. Therefore, breeding programs should consider GxE interaction. In Chapter 4, genetic parameters for log-transformed variance of deviations (LnVar), a good indicator of resilience, were estimated. Heritable genetic variation was found for LnVar and it was noted that LnVar is more expressed in the non-aerated pond compared to the aerated pond. Finally, in Chapter 5, we estimated genetic parameters for critical swimming speed (Ucrit) and the genetic correlation between Ucrit and harvest weight of fish raised in a non-aerated pond. Substantial heritable variation was found for Ucrit. The estimated genetic correlations between Ucrit early in life and HW after grow-out in non-aerated pond, and Ucrit and growth were negative, implying that Nile tilapia with higher Ucrit early in life had lower HW and growth later in life. In this Chapter 6, the findings from the previous chapters combined and discussed their implications for breeding programs.

## Contents

CHAPTER 1

# General introduction

## 1.1 Introduction

## 1.1. World aquaculture

From 2000 to 2018, total world aquaculture production increased by 42%, reaching an all-time high (FAO, 2020b). From 1995 to 2018, the trend of aquaculture fish production by percentage of world total was increasing in Africa and the Americas, and the trend was stable in Asia; however, the trend was decreasing in Europe (FAO, 2020a). In 2018, the value of aquaculture production was 139.7 billion USD and aquaculture employed 20.5 million people (FAO, 2020a). Aquaculture is the fastest-growing livestock production sector (Garlock *et al*., 2020). Freshwater fish aquaculture production increased by 131% between 2000 and 2018 (FAO, 2020b). Most aquaculture production comes from Asian countries, dominated by China, Indonesia and India (FAO, 2020b). Carps and tilapia are the dominant freshwater species groups.

## 1.2. Nile tilapia Aquaculture

Nile tilapia is the most important commercial fish species in tropical freshwater aquaculture, with an estimated global production of 4.8 million tons in 2018 (FAO, 2020a). Nile tilapia constitutes 8.3% of the total finfish produced in 2018, which makes it the third most-produced finfish next to Grass carp (*Ctenopharyngodon idellus*) (10.3%) and Silver carp (*Hypophthalmichthys molitrix*) (8.4%) (FAO, 2020a). Global Nile tilapia production increased by 19.6 times in 2019 compared to 1990 (Figure 1.1) (FAO, 2021). Nile tilapia production in 2019 increased from about 201 thousand tons to 2.9 million tones in Asia, from about 4.7 thousand tons to 436 thousand tons in Latin America and from about 27 thousand tons to 1.26 million tons compared to 1990 (Fig 1).

The feeding habit, high growth rate and disease tolerance of Nile tilapia have helped the production of this species to become widespread. Tilapias are herbivorous/omnivorous fish (El-Sayed, 2006), therefore, less wild fish is used in tilapia feed than in carnivorous fish feed (Naylor *et al*., 2021); and feeding tilapia is less costly than carnivorous fish. Nile tilapia is also hardy, resistant to disease and tolerates poor water quality (Bhujel, 2014), and is farmed under diverse production systems: extensive, semi-intensive, or intensive production systems. Extensive and semi-intensive Nile tilapia farms are managed by smallholder farms, while the intensive Nile tilapia farms are managed by big commercial companies.

Extensive production systems are characterized by the use of low stocking density, no or limited use of supplementary feed or fertilization as Nile tilapia can rely on

natural food. Semi-intensive production systems are characterized by moderate input use, pond fertilization using manures or inorganic fertilizers to boost natural food production, use of supplementary homemade or commercial feed, higher stocking density than extensive production systems, and Nile tilapia can be farmed in monoculture or polyculture system. Earthen ponds without aeration are most commonly used in both extensive and semi-intensive production systems. Absence of aeration results in recurrent high dissolved oxygen fluctuation during the day. Nile tilapia requires a normoxic environment (dissolved oxygen above 5 mg/L) for optimal growth. However, tilapia are known to tolerate dissolved oxygen levels, which often drop below 3 mg/L during nights.

Intensive production systems are characterized by high input use, high stocking density, use of a nutritionally complete pelleted diet, use of continuous aeration for an optimized environment. Intensive Nile tilapia production is practiced in raceways, cages and ponds. Extensive and semi-intensive production systems are probably the dominant production systems, but exact numbers are not available. Hereafter, both extensive production systems and semi-intensive production systems are referred to as smallholder production systems.

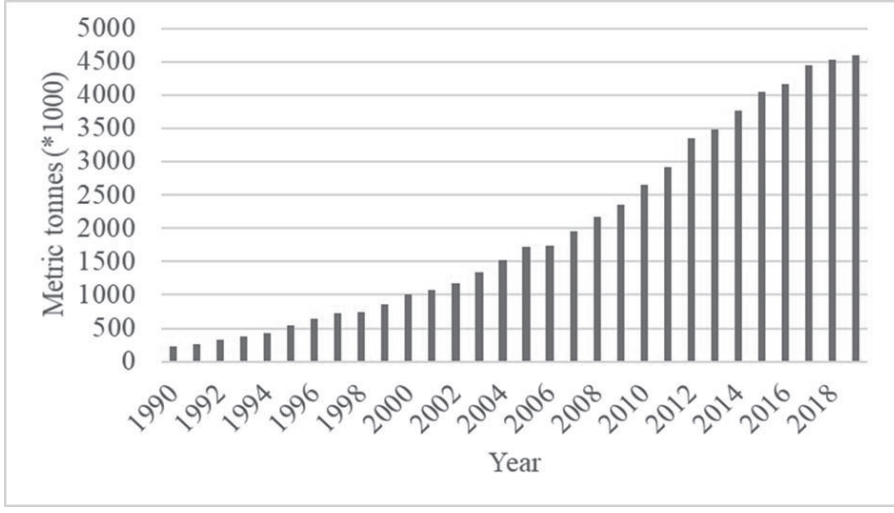


Figure 1.1 World tilapia production from 1990 to 2019 (FAO, 2021).

## 1.3. Nile tilapia selective breeding programs

Many selective breeding programs have been established for Nile tilapia. Until 2005, seventeen breeding programs were established for Nile tilapia (Neira, 2010),

for example, Genetically Improved Farmed Tilapia (GIFT) (Pullin *et al*., 1991; Eknath *et al*., 1993; Bentsen *et al*., 1998) and FaST(Bolivar, 1998), GenoMar Supreme Tilapia (Zimmermann and Natividad, 2004) and GET-EXCEL (Tayamen, 2004). Among the Nile tilapia selective breeding programs, the GIFT breeding program is the most important non-commercial breeding program. The GIFT project was started in 1988 in the Philippines and executed by the International Center for Living Aquatic Resources Management (ICLARM), now WorldFish, in collaboration with partner organizations (Pullin *et al*., 1991). The base population was formed from four wild stocks from Egypt, Ghana, Senegal and Kenya and four commercial Nile tilapia strains farmed in the Philippines (Pullin *et al*., 1991; Gjedrem, 2012). After six generations of selective breeding in the Philippines, WorldFish continued the selective breeding of the GIFT strain in Kedah State, Malaysia, from generation six after receiving 63 full sib groups of 35 fish each from the Philippines towards the end of 2000 and the beginning of 2001 (Ponzoni *et al*., 2011b). Currently, the GIFT strain has been selected for 20 generations in Malaysia and disseminated to more than 16 countries worldwide (Agha *et al*., 2018; WorldFish, 2021).

The Genetically Improved Farmed Tilapia (GIFT) breeding program benefited from the experiences gained in salmon breeding that started in 1971 in Norway (Gjedrem, 2012). Genetic gains in harvest weight ranging from 7-11% per generation over four to six generations of GIFT breeding program have been published (Khaw *et al*., 2008; Ponzoni *et al*., 2011a; Thodesen *et al*., 2011). After six generations of selection, the GIFT strain grew up to 85% faster than the fish used in the base population (WorldFish, 2021). The estimated selection response ranges from 10 to 15% in the last ten generations (Khaw, 2015). Recently estimated average selection response for GIFT strain was 7% for 17 generations, indicating continued response to selection (Benzie *et al*., 2021). Data from breeding programs conducted with other Nile tilapia strains by WorldFish in Abbassa, Egypt, indicate that on average 3% selection responses have been achieved for 12 generations (Benzie *et al*., 2021).

Other Nile tilapia selective breeding programs also reported considerable selection responses. In China, on average 11.4% (range 7.4 to 18.7%) selection response was recorded for Progift Nile tilapia body weight (Thodesen *et al*., 2011). For GenoMar Supreme Tilapia the average selection response over 11 generations was 17% (Gulzari, 2017).

All the Nile tilapia selective breeding is undertaken in optimal environmental conditions. However, there are large differences in productivity between the best performing farms and low performing farms. A mismatch between genotype and environment could lead to such differences. The GIFT strain has been selected under an optimal dissolved oxygen environment while smallholder production is undertaken in non-aerated earthen ponds. The dissolved oxygen (hereafter DO) differences between the selection and the smallholder production environment could lead to genotype-by-environment (GxE) interaction. That means the genetic gain achieved in the selection environment may not be fully achieved in the production environment.

Many studies have investigated GxE interaction in Nile tilapia between different environments: different fertilization and different feeding supplement (Eknath *et al*., 2007), fertilized pond with/without feed supplement, cage culture with feed supplement/commercial pellet feed and rice-fish culture (Bentsen *et al*., 2012) cage and pond environments (Khaw *et al*., 2012), low and high input environments (Trọng *et al*., 2013), monosex and mixed sex (Omasaki *et al*., 2016). In total 17 studies investigated GxE interaction between different environments (reviewed by Sae-Lim *et al*., 2016). Most of these studies concluded that genetic correlations were less than unity. However, none of these GxE studies investigated the effect of aeration.

DO is one of the limiting factors of productivity. Under climate change, DO is expected to decrease with increasing water temperature (Lennox *et al*., 2019). Increasing water temperature and dropping DO levels are expected to cause more stress and diseases are projected to become more frequent (Alders *et al*., 2021). Hypoxia can suppress growth and immunity in fish (Abdel-Tawwab *et al*., 2019). Therefore, optimal Nile tilapia farming requires appropriate levels of DO. However, most smallholder Nile tilapia farms do not use aerator which leads to recurrent hypoxia. Recurrent hypoxia could lead to poor performance of Nile tilapia. Genetic improvement of Nile tilapia has been carried out in an aerated environment (normoxic environment), mainly focussing on improving harvest weight. Given these challenges, Nile tilapia breeding programs need to improve Nile tilapia resilience to stressors such as diseases and tolerance to DO fluctuation.

Resilience is defined as the capacity of an animal to be minimally affected by perturbations or to quickly recover to the state it had before the perturbation (Colditz and Hine, 2016). A Log-transformed variance of deviations (LnVar) can be

used as an indicator of resilience and animals with better resilience show lower LnVar values (Berghof *et al*., 2019b). More resilient animals are expected to be less disturbed by perturbation and could grow more uniformly. More uniform fish growth could improve biomass estimation, feeding management, welfare and avoid size grading. More accurate biomass estimation could reduce feed wastage resulting from exaggerated biomass estimation. Resilience is favorably correlated with the survival and health of animals. Mulder *et al*. (2015) found a favorable genetic correlation between residual variance of piglet birth weight, an indicator of resilience, and survival at birth. The genetic correlation between LnVar of milk yield and traits such as health, ketosis, fertility and longevity were negative and favorable (Elgersma *et al*., 2018; Poppe *et al*., 2021). Therefore, selective breeding of Nile tilapia for lower LnVar could improve health, welfare and survival.

Different studies in dairy cattle, sheep, pigs and chicken found heritable variation for resilience based on repeated measurements (Elgersma *et al*., 2018; Berghof *et al*., 2019a; Putz *et al*., 2019; Dobrzański *et al*., 2020; Poppe *et al*., 2020; Garcia-Baccino *et al*., 2021; Moncur *et al*., 2021; Poppe *et al*., 2021), indicating resilience can be improved by selective breeding. However, we have not found any studies on resilience in Nile tilapia.

Recurrent hypoxia tolerance, one of the aspects of resilience, is also an important trait. Aerobic metabolism is 30 times more efficient than anaerobic metabolism and, therefore, hypoxia tolerance is an advantage (Richards, 2009). Better hypoxia tolerance could help fish to maintain aerobic metabolism and improve feed efficiency. In fish critical swimming speed may reflect the oxygen uptake efficiency. Vandeputte *et al*. (2016) found heritable genetic variance for swimming performance in European sea bass (*Dicentrarchus labrax*). Genetic parameter estimates for swimming performance and genetic correlation between swimming performance and production traits in Nile tilapia are not investigated so far.

## 1.4. Motivation and objectives of the study

Currently, many small- and medium-sized tilapia farms in developing countries still underperform in terms of feed efficiency, despite the use of genetically improved strains of tilapia such as GIFT. Productivity differences among many small- and medium-sized tilapia farms lead to a yield gap between the best performing and low performing farms. In aquaculture, growth, feed conversion ratio (FCR) and survival are the main determinants of productivity (Kankainen *et al*., 2012; de Verdal *et al*., 2018).

Reported survival in ponds varies considerably and range from 29 to 80% (Abdalla *et al*., 1996; Abdelghany and Ahmad, 2002; Rana and Hassan, 2013). Mortality at the later grow-out period is economically more important than mortality at the early grow-out stage. Typically, these losses only become evident at harvest, as fishes are grown in ponds or cages, and biomasses or standing stocks can only be estimated. Mortality leads to feed wastage, lower than estimated harvests and significant loss of revenues. The causes of mortality are to a large extent unknown and probably occur gradually over time. FCR in tilapia farms ranges from 1.5 to 2.5 (Rana and Hassan, 2013). Commercial harvest weights typically range from 800 to 1400 g in intensive production systems but harvest weights in smallholder production system do not exceed 200-300 g. Selective breeding has been a successful means of improving growth and FCR in Nile tilapia (Thodesen *et al*., 2011; Gulzari, 2017; de Verdal *et al*., 2018). Recurrent hypoxia in most smallholder Nile tilapia farms could lead to poor performance of Nile tilapia. A mismatch between breed and environment can also cause poor performance of Nile tilapia at the farm level, indicating the need for an optimal solution to alleviate the yield gap problem.

Therefore, the aim of this thesis was to optimise the breeding program of Nile tilapia for a smallholder production system, thereby contributing to closing the yield gap. The specific objectives were:
1) to quantify the effects of the most likely environmental and management factors on FCR, mortality and growth of Nile tilapia,
2) to investigate the presence of genotype by environment interaction between aerated and non-aerated ponds,
3) to estimate genetic parameters for resilience and
4) to estimate genetic parameters for swimming performance of Nile tilapia and to estimate the genetic correlation between swimming performance and production traits in aerated and non-aerated ponds.

## 1.5. Outline of this thesis

We started with a systematic quantitative literature review to identify the most important yield gap factors in Nile tilapia farming (chapter 2). The results of the systematic quantitative literature review confirmed that dissolved oxygen is a major environmental factor contributing to reduced growth and FCR. Therefore, we designed an experiment in which fish were raised in an aerated and a non -aerated pond, in order to estimate genotype by environment (GxE) interaction for different traits between aerated and non-aerated ponds. Our hypothesis was that there is

substantial GxE interaction for harvest weight and growth between aerated and non-aerated ponds (chapter 3).

One way to estimate the impact of the environment is to estimate resilience for growth (Berghof *et al*., 2019b). We, therefore, took repeated measurements of body weight and photograph of each fish overtime during the grow-out in the GxE experiment to calculate the variation in body weight growth trajectories, log-transformed variance of deviations (LnVar). The hypothesis was that aeration would affect resilience, expressed as fluctuation in individual growth rate (chapter 4).

To further investigate the relationship between growth and hypoxia tolerance, we designed a swim test where fish have swum until exhaustion in swim flume (Palstra, 2016). The maximum flow rate at which this is achieved can be used to calculate critical swimming speed ($U_{crit}$) (Brett, 1964). In chapter 5, we performed an experiment where fish were tested for $U_{crit}$ t, and subsequently raised to harvest weight in a non-aerated pond. The hypothesis was that $U_{crit}$ is heritable and that Nile tilapia with high oxygen uptake efficiency (O2UE) may perform better than Nile tilapia with low O2UE under non-aerated ponds.

Finally, in chapter 6 I combine the findings in chapter 2 to 4 and discuss the implications for breeding programs. I present breeding strategies that use the novel traits $U_{crit}$ and LnVar that can be used to close the yield gap with smallholder tilapia farmers.

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CHAPTER 2

# 2

# A systematic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia (*Oreochromis niloticus*)

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## Abstract

Productivity among small- and medium-scale tilapia farms varies considerably. The difference between the best performers and lower ones (yield gap), is affected by differences in growth rate and feed conversion ratio (FCR). FCR at the farm level is strongly influenced by survival of fish. In this study a systematic literature review of two databases (ASFA and CAB-Abstracts) identified 1973 potentially relevant articles. Data from 32 articles that met the inclusion criteria were analysed using linear mixed models for the most important factors with significant contributions to growth [investigated through analysis of the thermal growth coefficient (TGC)], survival and FCR of Nile tilapia. Increasing crude protein (CP), dissolved oxygen (DO) and pH significantly decreased FCR and increased TGC. Increasing stocking weight (SW) significantly improved both FCR and survival. Temperature had the largest effect on FCR followed by DO, pH and CP. DO had the largest effect on TGC followed by CP and pH. This study confirms that the optimal rearing temperature for Nile tilapia is between 27 and 32℃. Improving management to optimize DO (>5 mg/L), stocking density (3 – 5 fish/$m^2$), SW (>10g) and CP (25 − 30%) will improve performance and survival in small- and medium-scale tilapia farming. However, it is hard to influence temperature in ponds and cages while DO is largely influenced by aeration. Since many small- and medium-sized farms do not have aeration, these major tilapia farming systems could benefit from genetically improved strains selected for resilience to highly fluctuating diurnal temperature and DO levels.

Key words:, feed conversion efficiency, growth, survival, tilapia, yield gap

## 2.1 Introduction

The supply of fish for human consumption has been increasing at a rate of 3.2% per year since the 1970s until 2013. Aquaculture made a substantial contribution to this increase, with inland finfish farming contributing 65% of the increase in fish production from 2004 to 2014 (FAO 2016). Among the finfish, Nile tilapia *(Oreochromis niloticus)* ranked second in terms of production volume next to carps (grass carp, silver carp and common carp) with a total production volume of 3.7 million tons worth about 6 billion USD (FAO 2016). Nile tilapia is farmed in more than 80 countries and in different production systems ranging from artisanal to intensive systems (Norman-López and Bjørndal 2009). Tilapia is an important fish species for home markets in Asia, South America and Africa; the United States of America is the major export market for tilapia (FAO 2016). Therefore, many selective breeding programs have been established for Nile tilapia (Neira 2010) including an important non-commercial breeding program by WorldFish that developed the genetically improved farmed tilapia (GIFT). The GIFT strain has been disseminated to many countries (Komen and Trong, 2014). According to Neira (2010), 10 out of 17 Nile tilapia breeding programs had used the GIFT strain as their base population.



Nile tilapia production systems can be classified in terms of input utilisation as extensive, semi-intensive and intensive farming systems. The earthen-pond production systems are the dominant ones practiced by small- and medium-sized tilapia farms. Such farms typically produce fish of 200 - 500 grams weight targeting local markets. Larger fish with harvest weights above 800 grams are produced by large farms that mostly use larger ponds with aeration, or cages in lakes and reservoirs (Omasaki *et al*. 2016b, Hoong Yip Yee, pers. comm., 2016). Currently there is big a difference in productivity among many small- and medium sized tilapia farms. The difference in productivity between the best performing farms and low performing farms is defined as a yield gap, the difference between achieved production and that which is possible with optimal management. Many factors can contribute to differences in productivity but all have their action ultimately in their effects on growth, survival and feed efficiency, and this can be summarised as a difference in feed conversion ratio (FCR). There are large differences in FCR and survival among many small- and medium-sized tilapia farms.

FCR at the level of production units is defined as the ratio of the total feed given divided by total biomass harvested. FCR is determined by individual feed efficiency and survival, because fish that die during the grow-out period eat feed until death, but do not contribute to the total biomass harvested. Reported FCR values for tilapia vary widely, ranging from 1.5 to 2.5 in pond environments and from 1.0 to 1.71 in cage environments (Rana and Hassan 2013). Thoa *et al*. (2016) reported FCR values of 1.08 and 1.89 in freshwater and saline water pond environments, respectively. FCR is considered acceptable when it is not higher than 2 (Craig 2009) but the acceptable level can vary with the feed price. Feed cost is the major cost in fish farming (Craig 2009, El-Sayed 1999) representing over 50% of the variable costs during the grow-out period (El-Sayed 1999). In places where the feed price is high, a small increase in FCR could considerably increase the variable cost. Therefore, underperformance in terms of FCR is a major concern for aquaculture as it strongly and negatively affects the profitability of fish farms.

Both primary determinants of FCR at the production unit level, mortality and individual differences between fish in converting feed to biomass, are strongly influenced by the environment (de Verdal *et al*. 2018). Mortality, especially late mortality, is an important determinant of FCR. Rates of mortality for Nile tilapia vary considerably, with 20 - 71% mortality being reported for Nile tilapia reared in fertilised ponds with or without supplementary feeding (Abdalla *et al*. 1996; Abdelghany and Ahmad 2002). According to Rana and Hassan (2013) the reported mortality varies between 25 and 60% in pond environments. Trọng *et al*. (2013) reported a mortality rate of 71-72% for the cage culture environment, 48% for the pond nucleus environment and 32% in the polyculture production environment in Vietnam. The economic effect of mortality depends on the stage during which fish mortality happens. Mortalities occurring during the later stages of the grow-out phase have the largest economic impact due to the accumulated cost of production. The amount of feed delivered at any one time is usually based on the estimated standing stock of fish and the FCR is measured based on the amount of feed fed and the biomass harvested. Overestimating the standing stock will increase the feed waste, which has a negative effect on profit and environment, while underestimating leads to underfeeding of the fish and reduced production.

The wide range of FCR and mortality values reported indicate a large difference between the best and worst performing farms and suggest significant room for

improvement with respect to more efficient husbandry. The investment in genetic improvement programs designed to improve performance in farming systems is undermined by these inefficiencies. Investigating the factors that contribute to the reduced productivity of tilapia fish farms is critical to providing the information needed to tackle the yield gap problem. First, by determining whether husbandry approaches can be optimised, and second, for those aspects of the environment that cannot be managed, identifying whether farmed strains can be genetically improved to be more efficient in those environments.



Work over the last two decades has established some of the main parameters for optimising the environment for rearing tilapias (Popma and Masser 1999; El-Sayed 2006, Mjoun *et al*. 2010). However, there has been no comprehensive analysis of the actual performance of Nile tilapia in farm systems that provide the critical information as to how best to address the yield gap for this globally important aquaculture resource – either through improved husbandry or through selective breeding. The objective of this study was to quantify the effects of the most likely environmental and management factors on FCR, mortality and growth of Nile tilapia and to identify the most important of these factors associated with the yield gap.

## 2.2 Material and methods

### 2.2.1 Literature search

A systematic literature search was conducted for peer-reviewed journal articles that had been published in English in the ASFA (1971-2016) and CAB-Abstracts (1979-2016) databases on the 7$^{th}$ of July 2016. We used the following search terms and Boolean operators ("feed efficiency" OR FCE OR "feed conversion" OR FCR OR "growth rate" OR survival OR mortality) AND ("Nile tilapia" OR "*Oreochromis niloticus*"). Based on the above search terms, we found 889 and 1739 articles from ASFA and CAB-Abstract databases, respectively. The two searches were combined and duplicates were removed using EndNoteX7. This resulted in 1973 articles, which were then checked against the search terms in the title and abstract, which resulted in 140 eligible peer-reviewed articles. From these potentially relevant studies, 108 studies were excluded for one of the following reasons: i) because articles were not accessible (21 studies), ii) because they did not report a sufficient proportion of the variables included in the different models (20 studies), or iii) because studies were outside the scope of this review. Studies on the effect of

density on survival during transportation, lethal dose of salinity, compensatory growth with feed restriction and refeeding, sex reversal, or varying crude protein levels during the study period were considered as being outside the scope of the review (Figure 2.1). The data were extracted from the remaining studies for analysis.

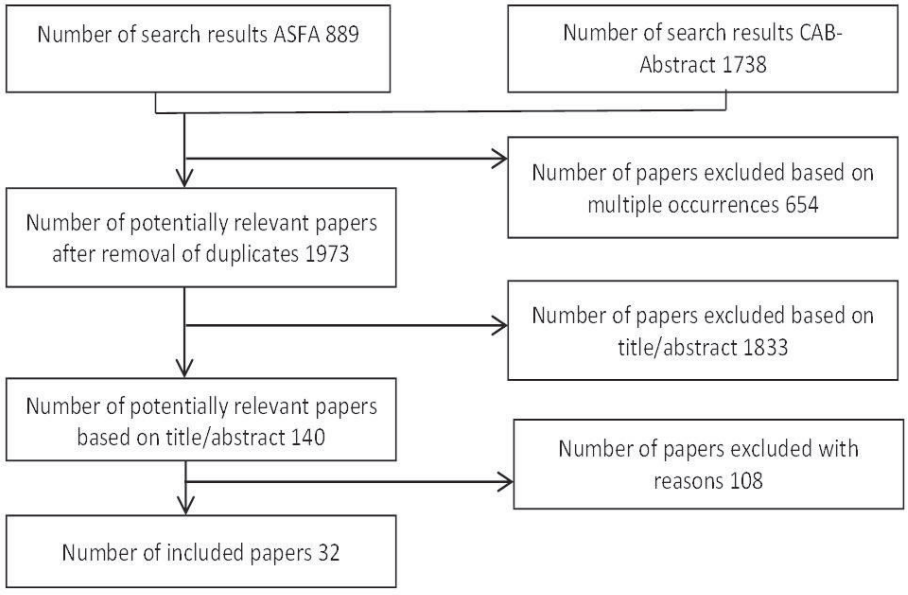


Figure 2.1 Flow diagram of article selection process.

### 2.2.2 Data extraction and statistical analysis

We extracted data on the following variables: study (since each study can be regarded as a separate element), "study length", which is the grow-out period studied, stocking density, feeding rate, feeding frequency, levels of crude protein (CP) in the diet expressed as percentage, stocking weight (SW), which is the weight at the beginning of the experiments, harvest weight (HW), water temperature, pH, dissolved oxygen (DO), salinity, ammonia, nitrate, nitrite, growth and survival. We also extracted FCR or calculated it as the inverse of total biomass harvested / total feed given. Based on the number of treatments within experiments in an article, multiple data records or results of treatments were extracted from each article. In

most of the studies, the numbers of fish used in the experiments or the standard errors were not reported and thus we gave equal weight to all the studies.

From the extracted variables CP, water temperature, pH and DO are environmental variables while the rest are management variables. FCR, survival and growth rate are the key determinants of productivity. To allow for comparisons across studies on growth rate, we calculated the thermal growth coefficient (TGC) as $\left[(\sqrt[3]{W_t} - \sqrt[3]{W_0})/(T \times t)\right] \times 1000$ where $W_t$ and $W_o$ are final and initial weights respectively, *T* is the average temperature during the growth period and t is the length of the growth period (Jobling, 2003). Therefore, the key traits analysed in this study were FCR, survival and TGC.



We first did a principal component analysis (PCA) using prcomp package in R software (R Core Team, 2015) to explore the explanatory variables. If variables were missing for some studies, we used the mean values for those variables and used all the 32 studies in the PCA. Next, we performed linear mixed models to estimate the effects of the explanatory variables on FCR, survival and TGC. The explanatory variables were study, study length, stocking density, SW, CP levels, DO, temperature, pH, feeding rate, feeding frequency, the quadratic terms of CP levels, DO, temperature and pH. Only a few studies reported salinity, ammonia, nitrate and nitrite and therefore the effects of these variables were not investigated. Linear mixed models were used to account for the variation in studies and study was fitted as a random variable, whereas the rest were fitted as fixed effects. All models were analysed using the lme4 package (Bates *et al*. 2015) for R software (R Core Team, 2015). The significance of fixed effects was based on the approximate Student's t-test (Bates *et al*. 2015). The non-significant effects were removed stepwise, leaving out the factor with the highest P-value.

## FCR

The majority of papers reported DO, but pH, feeding rate and feeding frequency were not reported in all the studies. Hence three separate analyses were undertaken, each with a different model.

Model 1 was used for studies that reported study length (L), stocking density (D), SW, CP, DO and temperature (T). The final analysis was based on 179 data records from 28 studies that report FCR:

$$FCR = \beta_0 + \beta_1 \times L + \beta_2 \times D + \beta_3 \times SW + \beta_4 \times CP + \beta_5 \times DO + \beta_6 \times T + \beta_7 \times CP^2 + \beta_8 \times DO^2 + \beta_9 \times T^2 + Study + \varepsilon \quad [1]$$

After removing the non-significant effects, the reduced model was:

$$FCR = \beta_0 + \beta_1 \times SW + \beta_2 \times CP + \beta_3 \times DO + \beta_4 \times T + \beta_5 \times T^2 + Study + \varepsilon \quad [1.1]$$

Model 2 used a subset of 23 studies out of the 27 used in model 1 that also reported pH which resulted in 141 data records:

$$FCR = \beta_0 + \beta_1 \times L + \beta_2 \times D + \beta_3 \times SW + \beta_4 \times CP + \beta_5 \times DO + \beta_6 \times T + \beta_7 \times pH + \beta_8 \times CP^2 + \beta_9 \times DO^2 + \beta_{10} \times T^2 + \beta_{11} \times pH^2 + Study + \varepsilon \quad [2]$$

After removing the non-significant effects, the reduced model was:

$$FCR = \beta_0 + \beta_1 \times CP + \beta_2 \times DO + \beta_3 \times T + \beta_4 \times pH + \beta_5 \times T^2 + Study + \varepsilon \quad [2.1]$$

Model 3 used a second subset of 11 studies out of the 27 used in model 1 that also reported feeding rate and feeding frequency which resulted in 67 data records:

$$FCR = \beta_0 + \beta_1 \times L + \beta_2 \times D + \beta_3 \times SW + \beta_4 \times CP + \beta_5 \times DO + \beta_6 \times T + \beta_7 \times Feeding\ rate + \beta_8 \times feeding\ freq. + \beta_9 \times CP^2 + \beta_{10} \times DO^2 + \beta_{11} \times T^2 + Study + \varepsilon \quad [3]$$

After removing the non-significant effects, the reduced model was:

$$FCR = \beta_0 + \beta_1 \times D + \beta_2 \times SW + \beta_3 \times CP + \beta_4 \times DO + \beta_5 \times T + \beta_6 \times Feeding\ rate + Study + \varepsilon \quad [3.1]$$

In all of three models, FCR equals feed conversion ratio, $\beta_0$ is the overall intercept, $\beta_1$to $\beta_{11}$ are the regression coefficients of the different explanatory variables on FCR, *Study* is a random study effect assumed to be normally distributed ($N(0, \sigma^2_{study})$, $\varepsilon$ is a residual random error assumed to be normally distributed ($N(0, \sigma^2_{\varepsilon})$, $\sigma^2_{study}$ is the variance due to study and $\sigma^2_{\varepsilon}$ is the residual variance.

## Survival

Model 4 was used to investigate the effect of study length, stocking density, SW, CP, DO and temperature on survival, based on 187 data records from 29 studies:

$$Survival = \beta_0 + \beta_1 \times L + \beta_2 \times D + \beta_3 \times SW + \beta_4 \times CP + \beta_5 \times DO + \beta_6 \times T + \beta_7 \times CP^2 + \beta_8 \times DO^2 + \beta_9 \times T^2 + Study + \varepsilon \quad [4]$$

The effects of CP, DO and temperature were not significant which led to the following reduced model:

$$Survival = \beta_0 + \beta_1 \times SW + Study + \varepsilon \quad [4.1]$$

$\beta_0$ is the overall intercept, $\beta_1$ is the regression coefficient of SW on survival, *Study* is a random study effect assumed to be normally distributed ($N(0, \sigma^2_{study})$, $\varepsilon$ is a residual random error assumed to be normally distributed ($N(0, \sigma^2_{\varepsilon})$, $\sigma^2_{study}$ is the variance due to study and $\sigma^2_{\varepsilon}$ is the residual variance.

A few studies on survival reported pH, feeding rate and feeding frequency, hence a separate set of analyses was done to investigate the effect of these explanatory variables, but none of them were significant and details of these models are not presented here.

## TGC

Model 5 was used to investigate the effect of study length, stocking density, SW, CP and DO on TGC. This model was fitted on 192 data records from 29 studies that reported TGC:

$$TGC = \beta_0 + \beta_1 \times L + \beta_2 \times D + \beta_3 \times SW + \beta_4 \times CP + \beta_5 \times DO + \beta_6 \times CP^2 + \beta_7 \times DO^2 + Study + \varepsilon \quad [5]$$

After removing the non-significant effects, the reduced model was:

$$TGC = \beta_0 + \beta_1 \times L + \beta_2 \times D + \beta_3 \times CP + \beta_4 \times DO \times + Study + \varepsilon \quad [5.1]$$

Model 6 was applied to a subset of 23 studies out of the 29 studies used in model 5 that also reported pH resulted in 155 data records:

$$TGC = \beta_0 + \beta_1 \times L + \beta_2 \times \mathrm{D} + \beta_3 \times SW + \beta_4 \times CP + \beta_5 \times DO + \beta_6 \times \mathrm{pH} + \beta_7 \times CP^2 + \beta_8 \times DO^2 + \beta_9 \times pH^2 + Study + \varepsilon \quad [6]$$

After removing the non-significant effects, the reduced model was:

$$TGC = \beta_0 + \beta_1 \times D + \beta_2 \times CP + \beta_3 \times DO + \beta_4 \times pH + Study + \varepsilon \quad [6.1]$$

Few studies reported feeding rate and feeding frequency together with TGC, hence we did a separate set of analyses, each with a different model, to investigate the effect of these variables on TGC.

Model 7 used another subset of 14 studies from model 5 that reported feeding rate and feeding frequency in addition to the other variables fitted in model 5, which resulted in 86 data records:

$$TGC = \beta_0 + \beta_1 \times \mathrm{L} + \beta_2 \times D + \beta_3 \times \mathrm{SW} + \beta_4 \times CP + \beta_5 \times DO + \beta_6 \times \text{feeding rate} + \beta_7 \times f\text{eeding freq.} + \beta_8 \times \mathrm{CP}^2 + \beta_9 \times DO^2 + Study + \varepsilon \quad [7]$$

After removing the non-significant effects, the reduced model was:

$$TGC = \beta_0 + \beta_1 \times feeding\ rate + Study + \varepsilon \quad 7.1]$$

With TGC being thermal growth coefficient, $\beta_0$ is the overall intercept, $\beta_1$ to $\beta_9$ are the regression coefficients of the different variables on TGC, *Study* is a random effect assumed to be normally distributed ($N(0, \sigma^2_{study})$, $\mathcal{E}$ is a residual random error assumed to be normally distributed ($N(0, \sigma^2_{\varepsilon})$, $\sigma^2_{study}$ is the variance due to study and $\sigma^2_{\varepsilon}$ is the residual variance.

The studies used in each model are given in Appendix 1.

## 2.3 Results

### 2.3.1 Principal component analysis

The first two principal components explained 42% of the variation in the whole data set. The correlations among DO, pH and feeding rate were positive. Stocking density and temperature were negatively correlated with DO, pH and feeding rate, whereas SW was negatively correlated with CP, DO, pH and feeding rate. Study length was negatively correlated with CP, feeding rate and DO (Figure 2.2).

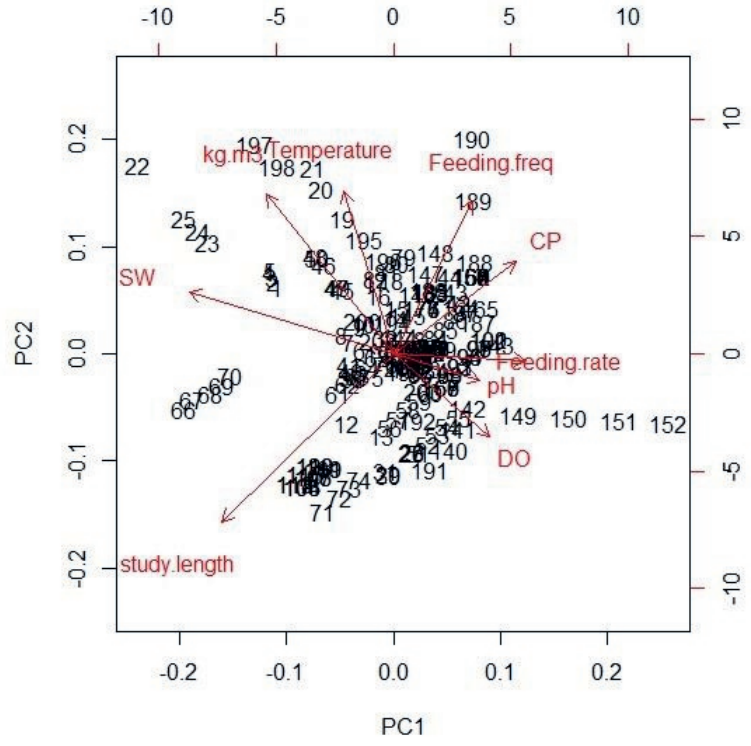


Figure 2.2 Loading plot from principal component analysis of all the data points from 32 studies.

## 2.3.2 Feed conversion ratio

The linear effects of CP, DO and temperature on FCR were significant in all three models (1, 2 and 3, $P < 0.05$, Figure 2.3a), whereas the quadratic term of temperature was significant in model 1 and 2 but not in model 3 when corrected for feeding rate (Table 2.1). The positive quadratic term of temperature in models 1 and 2 indicated that the relationship between FCR and temperature was not linear as demonstrated clearly in Figure 2.3b. The FCR was above 2.0 when the temperature was below 26℃ and above 33℃. Optimum FCR was between 27℃ and 32℃. FCR increased dramatically when the temperature drops below 25℃ and reaching 4.4 at 20℃ .

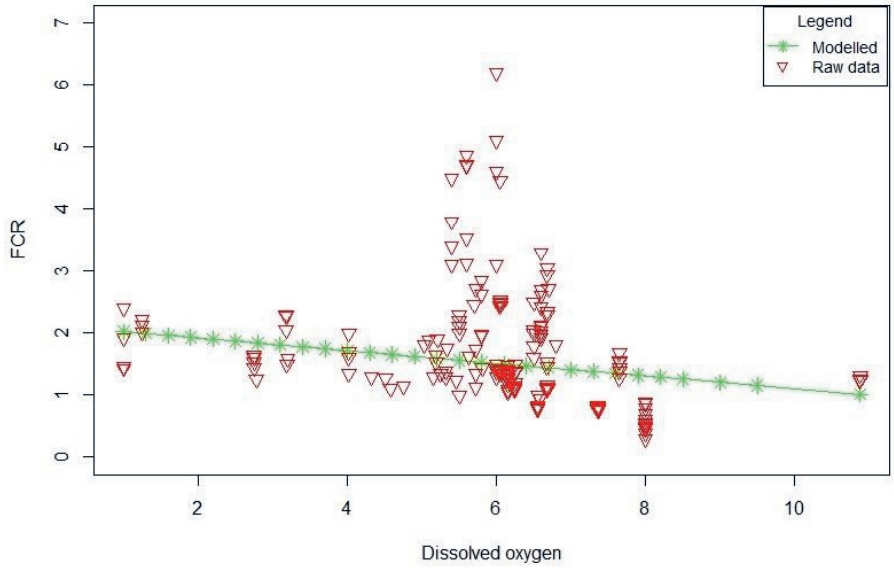


**Figure 2.3a** The effect of dissolved oxygen on FCR calculated based on the coefficient estimates from Table 1 model 1 and median values (SW=9.3, CP=34% and Temp.=28℃) for other variables. The linear equation is: $FCR = 32.4 + 0.003 \times 9.3 - 0.029 \times 34 - 0.102 \times DO - 1.99 \times 28 + 0.034 \times 28^2$.

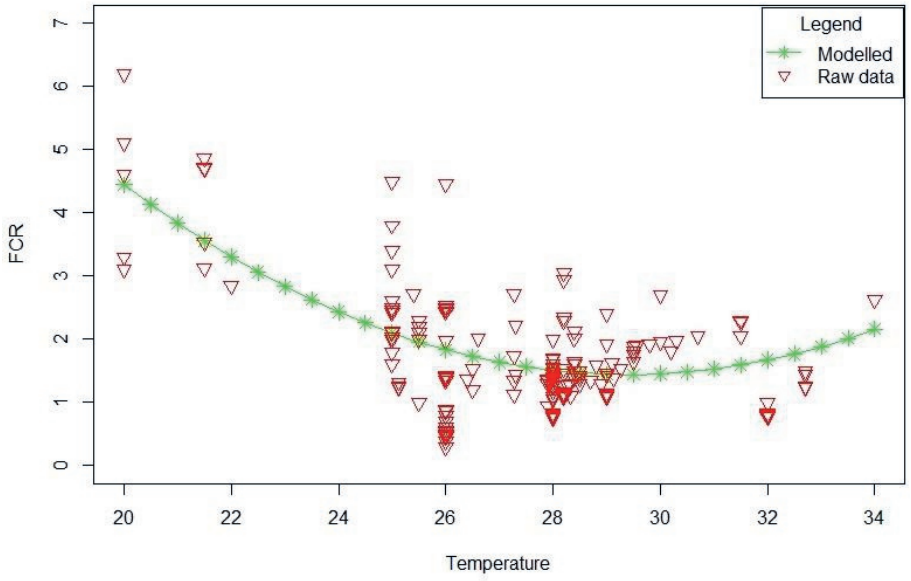


**Figure 2.3b** The effect of temperature on FCR calculated based on the coefficient estimates from Table 1 model 1 and median values (SW=9.3, CP=34%, and DO=6.05) for other variables. The linear equation is: $FCR = 32.4 + 0.003 \times 9.3 - 0.029 \times 34 - 0.102 \times 6.05 - 1.99 \times Temp. + 0.034 \times Temp.^2$.

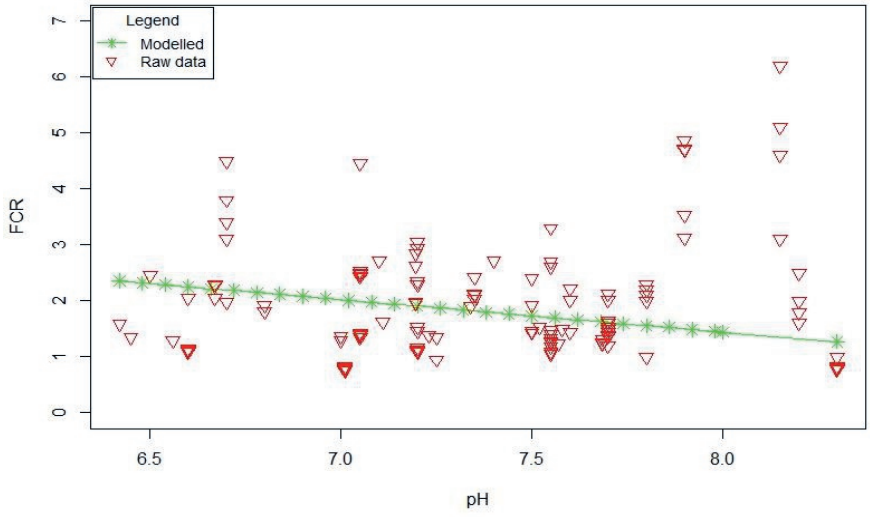


**Figure 2.3c** The effect of pH on FCR calculated based on the coefficient estimates from Table 1 model 2 and median values (CP=31%, DO=6.05 and Temp.=28℃) for other variables. The linear equation is: $FCR = 38.615 - 0.034 \times 31 - 0.101 \times 6.05 - 2.107 \times 28 - 0.579 \times pH + 0.036 \times 28^2$.

Table 2.1 Regression coefficient estimates ± standard errors given to one decimal place for factors that affect FCR for reduced Models 1, 2 and 3.

| | Model 1 28 (179)† | | Model 2 23 (141)† | | Model 3 11 (67)† | |
|---|---|---|---|---|---|---|
| Parameters | Parameter Range | Coefficient ± S.E. | Parameter Range | Coefficient ± S.E. | Parameter Range | Coefficient ± S.E. |
| Intercept | | 32.4±3.5*** | | 39.0±4.3*** | | 8.7±1.5*** |
| Stocking density (kg $m^{-3}$) | | | | | 0.003-22.0 | 0.1±0.0* |
| SW (g) | 0.012 – 311.1 | 0.0±0.0*** | | | 0.012–110.2 | -0.0±0.0* |
| CP (%) | 15-50.7 | -0.0±0.0** | 15–50.7 | -0.0±0.0*** | 17.2–50.7 | -0.0±0.0*** |
| DO (mg $L^{-1}$) | 1-11.1 | -0.1±0.0** | 1–10.9 | -0.1±0.0** | 3.2–10.9 | -0.2±0.1* |
| Temperature (°C) | 20-34 | -2.0±0.3*** | 20 – 34 | -2.2±0.3*** | 20–31.5 | -0.2±0.1*** |
| pH | NA | NA | 6.42 – 8.3 | -0.6±0.2*** | NA | NA |
| Feeding rate (% of body weight) | NA | NA | NA | NA | 2–60 | 0.1±0.0*** |
| Temperature$^2$ | | 0.0±0.0*** | | 0.0±0.0*** | | |
| Study variance | | 0.112 | | 0.118 | | 0.050 |
| Residual variance | | 0.248 | | 0.289 | | 0.337 |

†The number of studies and data records (in parentheses) utilized in each model are given below the model number and the studies are listed in detail in Appendix 1. Significance levels are indicated as * P<0.05, ** P<0.01 *** P<0.001.

Increasing levels of CP (15 − 50.7%) and DO (1 − 11.1mg/L) decreased FCR in all three models ($P < 0.05$, Table 1, Figure 2.3a), as did increasing pH (6.42 − 8.3) in model 2 ($P < 0.001$, Table 2.1, Figure 2.3c). Other variables tested in more than one model did not show a consistency of response or were not significant. FCR increased significantly with increasing stocking density when corrected for feeding rate in model 3 ($P = 0.017$), but was not significant ($P > 0.05$) in model 1 and 2. The effect of SW on FCR was significant and positive (0.003, $P < 0.001$) in model 1, not significant in model 2 ($P = 0.084$) but significant and negative (-0.016, $P = 0.017$, Table 1) when corrected for feeding rate in model 3. In model 1 and 2 the SW range is similar while in model 3 it is much smaller. The difference in the sign of coefficients of SW in model 1 and 2 is most likely due to the difference in SW ranges in the two models (0.003 – 311g and 0.012 – 110g, Table 1). The effect of feeding frequency, the quadratic terms of CP levels, DO and pH on FCR were not significant ($P > 0.05$) in any of the three models.

In summary, FCR decreased with increasing CP, DO and pH, and was optimal in a temperature range from $27.0 - 32.0℃$. Results were inconsistent for stocking density and SW. Among the environmental variables, temperature had the largest effect on FCR followed by DO and pH.

### 2.3.2 Survival

The analysis of model 4 showed a significant effect of only SW ($P = 0.025$, the linear equation is: $Survival = 89.767 + 0.03 \times SW$) on survival and no significant effect of any of the other variables ($P > 0.05$). Survival increased by 0.03% per gram increase in SW. Increasing stocking weight from 5g to 50 g would improve survival by 1.4% (Figure 2.4). The effects of feeding frequency, feeding rate and pH on survival were not significant for the range of values investigated (results not shown).

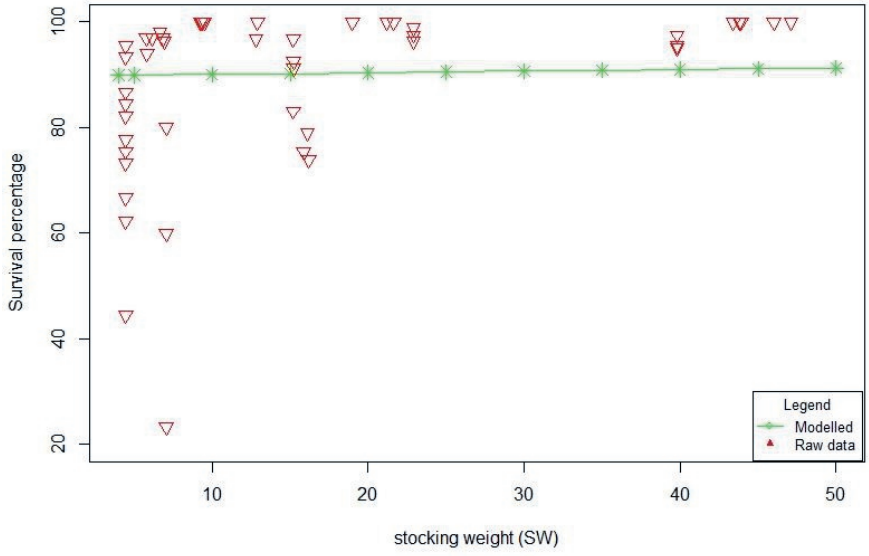


**Figure 2.4** The effect of stocking weight on survival. The fitted line was based on the estimated coefficients from model 5 and varying stocking weight from 4 to 50g. The resulting equation is: *Survival=89.767+0.03 × stocking weight*.

## 2.3.3 Thermal growth coefficient (TGC)

TGC increased with increasing levels of CP (15 − 50.7%) and DO (1 − 11.1mg/L) tested in models 5 and 6 ($P < 0.05$, Tables 2.2, Figure 2.5a) but when corrected for feeding rate in model 7 these effects became not significant. TGC increased with increasing pH (6.42 − 8.2) in model 6 ($P = 0.001$, Table 2, Figure 2.5b) and feeding rate (2 − 60%) in model 7 ($P = 0.030$, the linear equation is: $TGC = 0.611 + 0.01 \times feeding\ rate$).

Increased stocking density decreased TGC significantly in models 5 and 6 ($P < 0.05$) but not in model 7 ($P > 0.05$) which included feeding rate. The effect of study length on TGC was significant in model 5 ($P < 0.001$), but not in models 6 and 7 ($P > 0.05$). The effect of feeding frequency and the quadratic term of CP levels on TGC were not significant ($P > 0.05$). In summary, TGC increased with increasing CP, DO and pH and decreased with increasing stocking density and study length, although not in every analysis. Among the environmental variables DO had the largest effect on

TGC while, as expected, feeding rate had the largest effect on TGC from the management variables investigated.

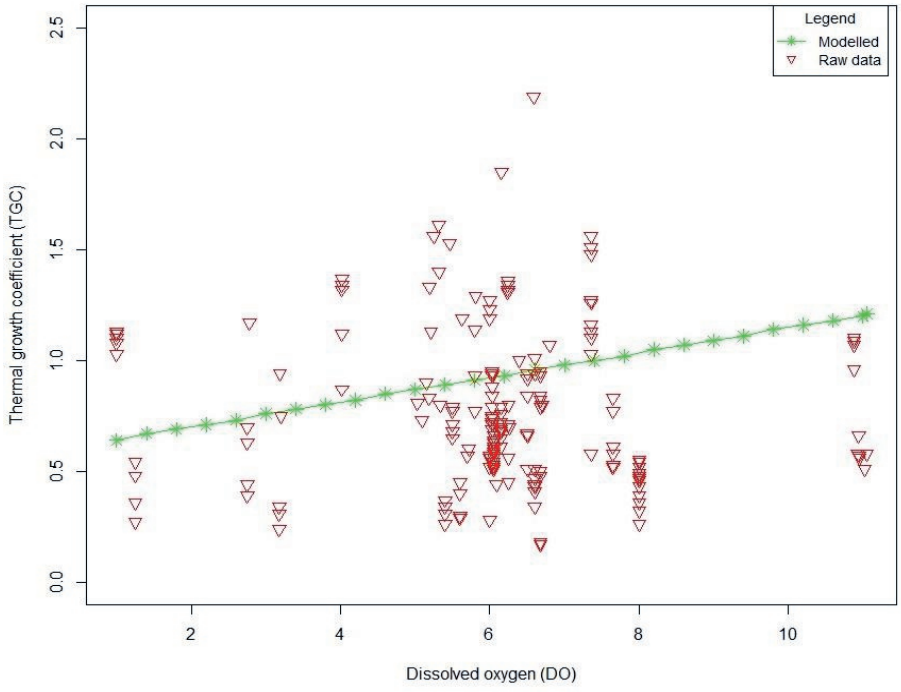


Figure 2.5a The effect of dissolved oxygen on TGC. The modelled data was calculated based on the coefficient estimates from Table 2 model 5 and median values (study length=70days, stocking density=0.894, CP=34% and Temp.=26°C) for other variables. The resulting linear equation is: $TGC = 0.436 - 0.003 \times 70 - 0.014 \times 0.894 + 0.011 \times 34 + 0.056 \times DO$.

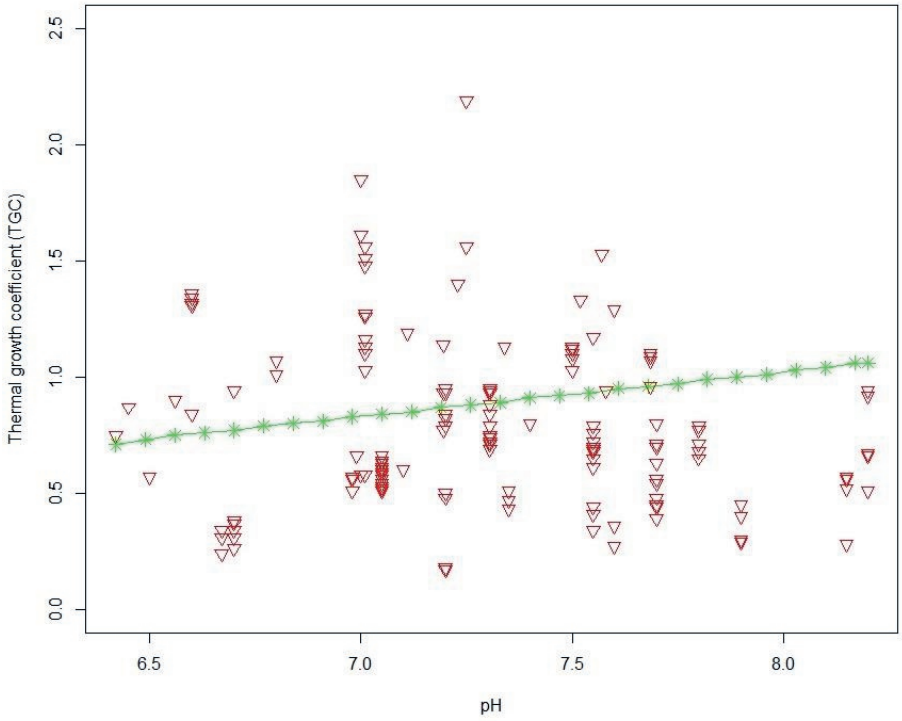


Figure 5b The effect of pH on TGC. The modelled data was calculated based on the coefficient estimates from Table 2 model 6 and median values (stocking density=0.894 and CP=34%) for other variables, resulting in the following equation: $TGC = -1.128 - 0.011 \times 0.894 + 0.01 \times 34 + 0.047 \times DO + 0.191 \times pH$.

Table 2.2 Regression coefficient estimates ± standard errors given to one decimal place for factors that affect TGC for Models 5 and 6. The number of studies and data records (in parentheses) utilized in each model are given below the model number and the studies are listed in detail in Appendix 1. Significance levels are indicated as * P<0.05, ** P<0.01 *** P<0.001.

| | Model 5 29 (192) | | Model 6 24 (155) | |
|---|---|---|---|---|
| Parameters | Parameter Range | Coefficient ± S.E. | Parameter Range | Coefficient ± S.E. |
| Intercept | | 0.4±0.2* | | -1.1±0.4* |
| Study length (days) | 25-196 | -0.0±0.0* | | |
| Stocking density (kg $m^{-3}$) | 0.003-41.4 | -0.0±0.0** | 0.003-39.0 | -0.0±0.0* |
| CP (%) | 15-50.7 | 0.0±0.0** | 15–50.7 | 0.0±0.0* |
| DO (mg $L^{-1}$) | 1-11.1 | 0.1±0.0** | 1–11.1 | 0.1±0.0** |
| pH | NA | NA | 6.42 – 8.2 | 0.2±0.1*** |
| Study variance | | 0.158 | | 0.200 |
| Residual variance | | 0.027 | | 0.024 |

## 2.4 Discussion

The main environmental and management factors influencing survival, FCR and growth of Nile tilapia in the 32 papers identified in a systematic literature survey were DO, temperature, pH, CP, SW, feeding rate and stocking density. Ammonia, nitrite, nitrate and salinity are important water quality parameters worth of inclusion in the analysis but data on these parameters were only available in few studies and therefore these parameters were not investigated. We discussed the main environmental and management factors influencing yield gap focusing mainly on pond production which is the predominant production system. The PCA analysis showed a correlation between explanatory variables. Pearson correlations between the explanatory variables were non-significant to weak or moderate correlations. The highest correlation between stocking density and stocking weight was 0.57 ($P <$ 0.01). Using median values of the significant variables and coefficients from table 2.1, model 1 and varying DO from the lowest values to the highest value improved FCR by 50%. Using median values of the significant variables and coefficients from table 1.1, model 1 and varying temperature from 20 to 29.5°C improved FCR by 68%. Using median values of the significant variables and coefficients from table 2.1, model 2 and varying pH from the lowest values to the highest value improved FCR by 46%. Using the median values of the significant variables from table 2.2, model 5 for DO and from table 2, model 6 for pH and varying DO and pH levels from minimum to maximum improved TGC by 88 and 52%, respectively (Figure

2.5a and 2.5b). These results are now discussed with a view to determine whether changes to husbandry practices can reduce the yield gap or whether it is possible to provide solutions through selective breeding for those variables that are difficult or impossible to control in given farming systems.

## 2.4.1 Tilapia farmers practice and the effects of husbandry management

### 2.4.1.1 Stocking weight and study length

In this study, we found significant effects of stocking weight (SW) on FCR, when corrected for feeding rate, and on survival, with survival (Figure 2.4) and FCR (Figure 2.6) increasing with increasing SW. It is clearly seen from Figure 2.4 that increasing SW increased survival in the range of SW 4 to 10 g, whereas the relationship looks like a sigmoid curve when considering the whole range from 4 to 50 g. Fessehaye *et al*. (2006) found significant mortality due to size dependent cannibalism for Nile tilapia weighing 0.03 to 15.08 g. They found a sigmoid relationship between predator to prey weight ratio and the probability of prey being killed. This would explain the sigmoid relationship between SW and survival. Stocking fish larger than 10g and graded for size uniformity could help to avoid size dependent cannibalism at smaller SW. The ranges of stocking density tested in our models were from 0.003 to ~22 – 41 $kgm^{-3}$. When keeping DO constant at 3 mg/L or 5 mg/L and varying stocking density from 1 − 20 fish per cubic meter, FCR and TGC hardly changed indicated by the almost flat lines (Figure 2.7 and 2.8). However, when keeping stocking density constant and increasing DO from 3 to 5 mg/L, FCR reduced from 2.3 and 2.4 to 2.0 and 2.1 and TGC increased from 0.77 to 0.88 (Figure 2.7 and 2.8).

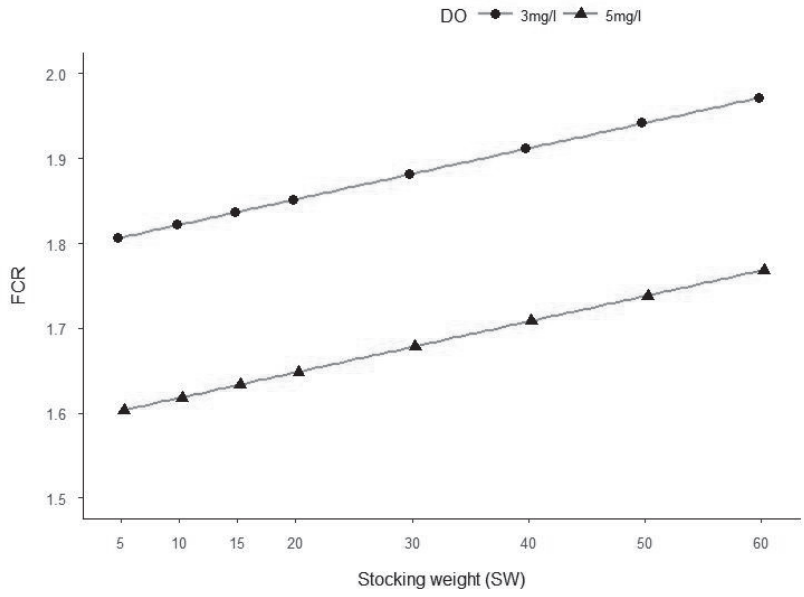


**Figure 2.6** The effect of SW and dissolved oxygen on FCR using coefficients from Table 1, model 1 and the median values (CP=34%, T=28℃) for the variables while varying SW from 5 to 60g and fixing dissolved oxygen (DO) at $3\text{mgl}^{-1}$(hypoxia) or $5mgl^{-1}$ (normoxia) ($FCR = 32.4 + 0.003 \times SW - 0.029 \times 34 - 0.102 \times DO - 1.999 \times T + 0.034 \times T^2$).

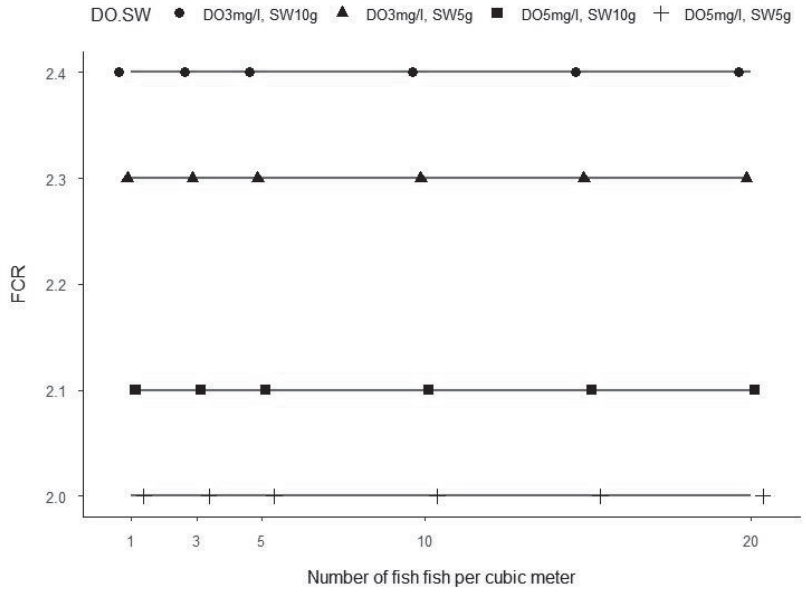


**Figure 2.7** The effect of stocking density on FCR in low oxygen (3mg/l) and high oxygen (5mg/l) levels. FCR was calculated based on the coefficient estimates from Table 1 model 3 and varying the density level for 5g and 10g fish, fixing dissolved oxygen level to 3 or 5mg/l and median values (stocking density=0.06, CP=34%, Temp.=26℃ and feeding rate=4% of body weight) for the other variables ($FCR = 8.728 + 0.097 \times 0.06 - 0.016 \times SW - 0.048 \times 34 - 0.165 \times DO - 0.163 \times 26 + 0.047 \times 4$).

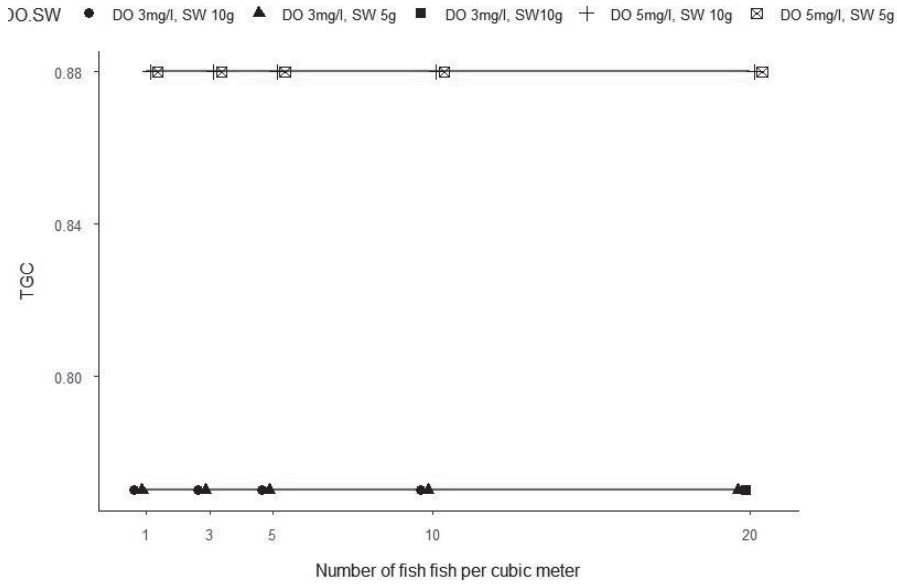


**Figure 2.8** The effect of stocking density on TGC in low oxygen (3mg/l) and high oxygen (5mg/l) levels. TGC was calculated based on the coefficient estimates from Table 2.2 model 5 and varying the density level for 5g and 10g fish, fixing dissolved oxygen level to 3 or 5mg/l and median values (study length=70, CP=34%) for the other variables ($TGC = 0.436 - 0.003 \times 70 - 0.014 \times D + 0.011 \times 34 + 0.56 \times DO$).

Under smallholder tilapia farm conditions diurnal DO fluctuation is very high. Therefore, stocking densities of 3 to 5 fish of size larger than 10g per square meter would give a better result than stocking smaller and/or more fish (Figure 2.6, 2.7 and 2.8).

The effect of study length on FCR was not significant while it was significant on TGC in model 5 while not significant in models 6 and 7. This is due to the fact that the analysis with model 5 has more data points with short study length. When two studies with short study length (El-Sayed and Teshima 1992, Tran-Duy *et al*. 2008) were removed, the effect of study length on TGC turned from statistically significant to non-significant. These studies are highly influential because the study length is relatively short at 25-30 days (average study length was 87.44 days) and the studies contributed 19 data points to the analysis.

Stocking density, corrected for feeding rate, had a significant effect on FCR (model 3), but it was not significant in model 1 and 2. It also had a significant effect on TGC (model 5 and 6). Increasing stocking density negatively affected both FCR and TGC. This agrees with what is generally observed in aquaculture (Ellis *et al*., 2002, Papoutsoglou *et al*., 2006, Li *et al*., 2012). Our estimates of the regression of stocking density on FCR (0.097) and stocking density on TGC (-0.014) suggests that increasing stocking density by one unit would lead to an increase in FCR by about 0.01 kg feed per kg biomass harvest and a reduction in TGC by 0.014.



Under small-scale tilapia production systems, stocking density, number of fish per square meter and stocking size differ from country to country. In Malaysia, five fish of 5 g per square meter are stocked (Azlan Bin Azizan, pers. comm., 2017); in China, 4 to 6 fish of 4 g on average are stocked per square meter in summertime, while in the wintertime they stock bigger fish, on average 18 g (Liu *et al*. 2013). In the Philippines, stocking density in extensive production systems is less than one fish of 10 to 20 g per square meter, in semi-intensive systems it is 1 to 5 and in intensive systems it is 5 to 10 fish of the same size, but in intensive systems using aeration, the preferred stocking size by farmers is five to twenty grams (Romana-Eguiaa *et al*. 2013). The growth period varies from two to nine months depending on the targeted market niche (Rana and Hassan 2013, Hoong Yip Yee, pers. comm., 2016) and therefore the length of the growth period, that is study length in this study, is not so much determined by its effect on FCR and TGC. The stocking density in Thailand is two to five fish (Bhujel 2013). The effect of stocking density is dependent on DO levels. Figure 2.7 and 2.8 suggest that 3 to 5 fish would give better FCR and TGC in a low oxygen environment. The above stocking densities used in different countries are in agreement with this study and will result in good FCR and TGC.

#### 2.4.2.1 Feeds and feeding

FCR (models 1, 2 and 3) and TGC (models 5 and 6) improved with increasing CP. Model 3 and model 7 showed that both FCR and TGC increased with increasing feeding rate. The effect of feeding rate on FCR and TGC is well described in the literature (e.g. review by de Verdal *et al*. 2017). As in terrestrial animals, protein plays a vital role in fish. It constitutes about 65 to 75% of fish body weight on dry matter basis (Halver and Hardy 2002). Fish require protein for growth, development and reproduction. Protein deficient feeds can negatively affect growth or lead to interruption of growth and loss of weight (Halver and Hardy

2002). Feed cost constitutes the major portion of the variable cost in fish farming (El-Sayed 1999) and protein is the most expensive feed ingredient. Profitability is a key factor in any commercial fish farming system. Therefore cost effective feed composition that can satisfy nutritional requirements and feed management that can optimize FCR and TGC is crucial.

The CP requirement for starter, grower and finisher fish is 30 to 35%, 30 to 32% and 28 to 30% respectively (FAO, 2018). Least-cost feed contains 20, 25 and 30% CP levels for finisher, grower and starter, respectively, at a feeding rate of 1.5 to 5% body weight and 3 to 4 feeds per day (Ng and Romano 2013). El-Saidy and Gaber (2005) found that the economic optimum is at 25% CP and feeding rate of 2% compared with 30% CP and 2% feeding rate for adult Nile tilapia reared in concrete tanks. According to a review by El-Sayed (2013), most smallholder farmers in sub-Saharan Africa fertilize their ponds to boost natural feed. In addition, some farmers use farm-made feed, cotton seed cake, wheat bran, rice bran or maize bran for supplementary feeding. In Thailand and the Philippines, smallholder tilapia farmers fertilize their ponds and use commercial feed, cereal brans, restaurant wastes or bakery wastes as supplementary feeding (Bhujel 2013, Romana-Eguiaa *et al*. 2013). According to a review by Rana and Hassan (2013), the CP content of tilapia feed used ranges between 16 to 32%. CP and feeding rate can be easily managed to optimize production and should be kept at the optimum level to maximize profit and minimize yield gap. The optimum feeding rate is the rate that gives the lowest FCR; this feeding rate is lower than the feeding rate required for maximum growth (for instance in salmon and trout Lovell, 1989). With respect to CP, in olive flounder, Kim *et al*. (2002) found that growth increased with increasing CP levels up to 55% and then decreased with further increase of CP. This would suggest a non-linear relationship between growth and CP. Therefore, we would expect also a non-linear relationship between FCR and CP. However, we found a linear relationship between CP and FCR within the range of values tested. Most of the studies used in this systematic review may have used CP levels close to the optimal or lower than the optimal levels with respect to FCR. If feed cost is increasing with CP, the economic optimum CP would be even lower than the CP that results in minimum FCR. Feed cost is the major cost in fish farming (Craig 2009, El-Sayed 1999) and among the feed ingredients CP is the most expensive. Therefore, feeding should be optimized to the level where marginal feed cost is equal to marginal revenues and where yield gap is minimal.

The effects of farm-made feeds, supplementary feeds and pond fertilization on yield gap were not investigated in our analysis. Farm-made feeds may vary in their nutrient content depending on the ingredients used. The CP content, CP source (NRC, 2011) and CP to energy ratio can affect feed efficiency (Kabir *et al*., 2019). Algae is a natural feed source for tilapia and the contribution of algae to tilapia growth is estimated to be between 40 and 68% in smallholder tilapia farms (Kabir *et al*., 2019). The amount of pond fertilization affects algae production in the pond. To reduce yield gap feeds should contain the optimum amount of nutrients and Bhujel (2014) recommends to maintain Secchi disc visibility at 30 - 40 cm depth for appropriate amounts of algae. If future studies include information on the type of farm made feeds, CP contents, CP source, CP to energy ratio and Secchi disc visibility, future meta-analyses could include these parameters to quantify the contribution of these factors to the yield gap, which would help in further minimizing the yield gap.



### 2.4.2 Environmental Factors

#### 2.4.2.1 Dissolved oxygen

In this study, we found significant effects of DO on FCR (models 1, 2 and 3) and on TGC (models 5 and 6) with FCR and TGC improving with increasing DO. The effect of the quadratic term of DO on FCR was not significant. Here we found only a significant linear association between DO and FCR, whereas the relationship might actually be curvilinear since there will be a DO level beyond which FCR will no longer improve. The reason that we did not find a curvilinear relation might be due to a lack of data points in the lower concentration range. Interestingly, DO had no significant effect on survival, at least not in the studies that were analysed in this paper. Our estimate of the regression of DO on FCR (-0.111) and DO on TGC (0.056) suggests that decreasing DO from the highest level investigated 11 mg/L to 3 mg/L, which is the minimum level required for tilapia production, would lead to an increase in 0.9 unit FCR (e.g. From 1 to 1.9) and to a reduction in 0.4 unit TGC. Using median values of the significant variables and coefficients from Table 2.1, model 2 and varying DO from 1 to 10.88 mg/L improved FCR by 50% (Figure 2.3a). Using coefficients and the median values of the significant variables from Table 2.2, model 5 and varying DO from 1 to 11.05 mg/L improved TGC by 88% (Figure 2.5a). The effect of DO on FCR is larger than the effect of pH, but lower than the effect of temperature whereas the effect of DO on TGC is larger than the effect of pH.

DO is one of the main limiting environmental variables that affect fish performance. Low DO affects feed intake negatively (Wang *et al*. 2009) and reduces digestibility (Tran-Duyen *al*. 2012). At high DO, feed assimilation is improved, which may be due to improved blood flow to the gastrointestinal tract (Axelsson *et al*. 2002) and lower energy cost of feed digestion and absorption of nutrients (Duan *et al*. 2011). Therefore, more energy is available for growth. Tran *et al*. (2016) found Nile tilapia performed significantly less in terms of final body weight, specific growth rate and FCR under hypoxia (3 mg/L) compared with under normoxia 5 mg/L which is 50% of saturation. They also found that hypoxia affected intestinal morphology negatively. Therefore, optimum DO is a very important environmental factor for improving FCR and TGC.

In non-aerated ponds, DO levels fluctuate during the day and will be somewhere < 1 – 15 mg/L with the highest values in the afternoon and the lowest values just before sunrise (Bhujel 2014). However, DO level should be kept at least 5 mg/L and when it drops to ≤ 3 mg/L, feeding should be stopped and remedial action should be taken to improve the DO levels (Stickney 2017). Pond aeration keeps DO at an acceptable level with minimal fluctuations. However, DO is often beyond control in many smallholder farms where aeration for fishponds is not available or too expensive.

In areas where aeration is available, ponds should be aerated during critical times of the day especially early in the morning and on cloudy days. Managing the algae load in the water to optimal levels also helps in minimizing the DO demand during the night and prevents a large drop of DO. Usually DO is not a problem in flowing rivers due to ample water movement, in lakes it can become a problem when it is highly eutrophic which results in algae bloom and hypoxia during nights. If aeration of ponds is not possible, it is clear that there is a need for fish that are resilient to low DO levels during parts of the day with low FCR and high TGC despite the extreme DO variation.

### 2.4.2.2 Temperature

Temperature had a significant effect on FCR (models 1, 2 and 3), while it had no significant effect on survival (model 4). The significant positive quadratic term clearly showed that the relationship between FCR and temperature is non-linear. FCR was optimum between 27℃ and 32.0℃ and increased significantly when the

temperature dropped below 25℃ reaching 4.4 at 20℃ (Figure 2.3b). Nile tilapia performs best in the upper end of the optimal temperature range of $27 - 32$℃, which is in agreement with older reports quoting 29 to 31℃ being the optimal temperature range for Nile tilapia (Popma and Lovshin 1996, Popma and Masser 1999). Note that the quadratic term of temperature was not significant in model 3 when accounting for feeding rate, which is most likely due to the fact that feeding rate was adjusted for temperature in the studies concerned. Using median values of the variables and coefficients from Table 2.1, model 1 and increasing temperature from 20 - 29.5℃ improved FCR by 68%, which was the highest effect compared with DO and pH (Figure 2.3b). Increasing temperature within the tolerable range increases appetite, food consumption rate and accelerates digestion of feed (Brett and Groves 1979, Jobling 1993). Management of water temperature in ponds and cages is not practical; thus, optimising temperature is not possible. Therefore, it can be concluded that it is important to select fish under conditions that are similar to the prevailing temperatures in commercial environments to optimize FCR.



### 2.4.2.3 pH

Our estimates of the regression of pH on FCR (-0.548) and pH on TGC (0.191) suggest that increasing pH by one unit from 6.42 to 7.42 would improve FCR by about 0.5 unit and TGC by 0.2 unit, respectively. Using median values of the significant variables and coefficients from Table 2.1, model 2 and varying pH from 6.42 to 8.3 improved FCR by 46%. Using values from Table 2.2, model 6 and the same approach as above, increasing pH from 6.42 to 8.2 improved TGC by 52%. The factors pH and DO had a comparable effect on FCR, whereas the effect of pH on TGC is half of the effect of DO on TGC. In line with our analysis, Popma and Masser (1999) found the best FCR and growth in a pH range from 7 to 9. However, un-ionized ammonia, which is toxic to fish, increases with increasing pH and water temperature (Randall and Tsui 2002). Therefore, in order to achieve best results pH should be maintained between 7 and 8. This can be practically achieved in ponds using lime (Calcium carbonate ($CaCO_3$)) (Lekang 2013).

Among the environmental factors pH can be easily managed to optimize growth, FCR and survival. Small-scale farmers manage water pH using lime, particularly under intensive pond production systems, while usually pH is not a problem for river cage production systems, where water exchange is sufficient to maintain pH

at optimum levels. Aeration can also help to reduce the amount of carbon dioxide that would otherwise interact with water and produce carbonic acid.

## 2.5 Implications for management and breeding

We conclude that CP, DO, water temperature, pH, stocking density and feeding rate are the most important variables to take into account to reduce the yield gap in tilapia farming. Ammonia, nitrite, nitrate, salinity and Secchi disc visibility are important water quality parameters but they were not investigated due to very few studies reporting these parameters. However, optimising DO, pH, stocking density and feeding rate positively affects ammonia, nitrite, nitrate, except for salinity. Low DO and high ammonia are not problematic in flowing rivers due to ample water movement. Salinity is a problem in areas with brackish water because Nile tilapia is a fresh water fish and less tolerant to salinity compared with other *Oreochromis spp*. (Watanabe *et al*., 1985). Temperature is practically beyond control in most farms. Tilapia farms should give emphasis to managing optimal stocking density and feeding rate. DO and pH are largely influenced by aeration and liming could improve pH when tilapia are grown in ponds. At present large numbers of small-scale farmers have no means to aerate their ponds, either because it is too expensive, or because they have no access to cheap electricity. Breeding programs should consider this. Selection for higher growth rate will increase feed intake and consequently oxygen consumption (Omasaki *et al*. 2017). As the selection environment is usually well managed, with optimal conditions in terms of DO, pH and CP, there is a risk for genotype by environment interaction (GxE) when improved strains are used in low-input ponds and a yield gap is expected because of lower production than what is genetically possible in an optimum environment.

In the GIFT breeding program, Ponzoni *et al*. (2011) reported a genetic gain of 10 - 15% per generation for growth. In the presence of GxE interaction, the same gain might not be attained in the production environment when DO and temperature are far from the optimum levels and create a large difference with the selection environment. Estimates of the degree of GxE for growth in Nile tilapia between different rearing environments are inconclusive (Sae-Lim *et al*. 2016). Charo-Karisa *et al*. (2006) found a low genetic correlation $(-0.27 \pm 0.69)$ for body weight of fry between ponds. Trọng *et al*. (2013) compared the growth of GIFT Nile tilapia reared in river cages, aerated nucleus ponds and non-aerated low-input ponds, and found a high genetic correlation (0.83) for daily growth coefficient (DGC). Eknath *et al*.

(2007) found high genetic correlations (0.76 − 0.99) among different pond environments and medium to high genetic correlation (0.36 − 0.82) between pond and cage environments. Bentsen *et al*. (2012) found high genetic correlations (0.53 − 0.99, mean = 0.89) for body weight between different environments. Robertson (1959) suggested that GxE interactions are biologically meaningful when the genetic correlation between environments is less than 0.8. GxE interactions with genetic correlations between environments of 0.8 or higher are considered not strong. However, if indeed the true genetic correlation is 0.8, it means that only 80% of the maximum possible genetic gain can be achieved in the production environment when selection is in the nucleus environment and information of only the selection environment is used in genetic evaluations (Mulder and Bijma 2005). Use of half-sib information from the production environment would reduce the loss in selection response (Brascamp *et al*. 1985, Mulder and Bijma 2005). Omasaki *et al*. (2016a) compared growth of Nile tilapia in a commercial monosex environment and a mixed sex nucleus environment and found significant GxE (genetic correlation=0.59) which was probably caused by the methyl-testosterone treatment to produce monosex fry. They recommend to use sib information from the monosex production environment, similar to the general recommendation by Mulder and Bijma (2005). Lower prices for genotyping may make it easier to include information of commercial animals in genetic evaluations and reduce the yield gap when using genomic selection (for instance see Mulder 2016).



## 2.6 Conclusion

We found that temperature had the largest effect on FCR followed by DO, pH and CP, whereas DO had the largest effect on TGC followed by CP and pH. Attempting tilapia farming in regions outside the optimal temperature range would have a negative effect on production efficiency unless the strains used are selected for such temperature range. Among the management variables, feeding rate had the largest effect on FCR and TGC followed by stocking density, study length and SW. Management could control these variables. Based on this review analysis we recommend optimizing management in terms of stocking density ($3 - 5$ fish/ $\mathrm{m}^2$), SW (>10 g), CP ($25 - 30\%$), DO (>5 mg/L) and pH ($7 - 8$) .This will improve FCR, survival and growth rate and reduce the yield gap in tilapia farming. Temperature has a very large effect on FCR, but it is hard to influence water temperature. DO is largely influenced by aeration when tilapia are grown in ponds. Since many small and medium sized farms do not have aeration, these major tilapia farming systems

could benefit from genetically improved strains selected for resilience to highly fluctuating diurnal temperature and DO levels.

## 2.6 Acknowledgements

This work is part of the first author's sandwich PhD study, funded by the Koepon Foundation whose support is acknowledged. The work was assisted through support provided to WorldFish by the European Commission-IFAD Grant Number 2000001539, the International Fund for Agricultural Development (IFAD) and the CGIAR Research Program on Fish Agrifood Systems (FISH).

## Appendix

Appendix 1 List of studies that were the source of data included for each analysis.

**Model 1**.

Investigated factor: FE; number of studies/number of data records: 28/179

Abdel-Tawwab et al. (2014)[1], Abdel-Tawwab et al. (2015)[1], Abou et al. (2007)[1], Al-Hafedh (1999)[1], Alhassan et al. (2012)[1, 2], Ali et al. (2008)[1], Azaza et al. (2008)[1], Azaza et al. (2013)[1], Azaza et al. (2015), Azevedo et al. (2015), Bahnasawy (2009)[, 2], Biswas and Takeuchi (2003)[1, 2], El-Sayed and Teshima (1992), El-Sherif and El-Feky (2009) ) [1, 2], Garcia et al. (2013)[1], Huang et al. (2015) [1, 2], Kamal and Mair (2005)[1], Kapinga et al. (2014)[1, 2], Kaya and Bilgüven (2015)[1, 2], Kpundeh et al. (2015)[2], Lanna et al. (2016)[1], Mohammad et al. (2015)[1], Mohammadi et al. (2014)[1], Ridha (2006)[2], Santiago et al. (1987)[1, 2], Sweilum et al. (2005)[1, 2], Tran-Duy et al. (2008)[1], Yi et al. (1996)[1]

**Model 2**

Investigated factor: FE; number of studies/number of data records: 23/141

Studies mentioned for model 1 in the cell above that are marked with superscript 1

**Model 3**

Investigated factor: FE; number of studies/number of data records: 11/63

Studies mentioned for model 1 in the cell above that are marked with superscript 2

**Model 4**

Investigated factor: Survival; number of studies/number of data records: 29/187

Abdel-Tawwab *et al*. (2014; 2015), Abou *et al*. (2007), Al-Hafedh (1999), Alhassan *et al*. (2012), Azaza *et al*. (2008), Azaza *et al*. (2015), Azevedo *et al*. (2015), Bahnasawy (2009), Biswas and Takeuchi (2003), El-Sayed and Teshima (1992), El-Sherif and El-Feky (2009), Garcia *et al*. (2013), García-Trejo *et al*. (2016), Huang *et al*. (2015), Kamal and Mair (2005), Kapinga *et al*. (2014), Kaya and Bilgüven (2015), Kpundeh *et al*. (2015), Lanna *et al*. (2016), Likongwe *et al*. (1996), Mohammad *et al*. (2015), Mustapha *et al*. (2014), Ridha (2006), Santiago *et al*. (1987), Sweilum *et al*. (2005), Tran-Duy *et al*. (2008), Yakubu *et al*. (2013), Yi *et al*. (1996)

**Model 5**

Investigated factor: TGC; number of studies/number of data records: 29/192

Abdel-Tawwab *et al*. (2014; 2015)[3], Abou *et al*. (2007)[3], Al-Hafedh (1999)[3], Alhassan *et al*. (2012)[3, 4], Ali *et al*. (2008)[3], Azaza *et al*. (2008)[3], Azaza *et al*. (2013)[3] , Azaza *et al*. (2015), Bahnasawy (2009)[3, 4], Biswas and Takeuchi (2003)[3, 4], El-Sayed and Teshima (1992), El-Sherif and El-Feky (2009)[3, 4], Garcia *et al*. (2013)[4], García-Trejo *et al*. (2016), Huang *et al*. (2015)[3, 4], Kapinga *et al*. (2014)[3, 4], Kaya and Bilgüven (2015)[3, 4], Kpundeh *et al*. (2015)[4], Lanna *et al*. (2016)[3], Likongwe *et al*. (1996)[3, 4], Mohammad *et al*. (2015)[3], Mohammadi *et al*. (2014)[3], Ridha (2006)[4], Santiago *et al*. (1987)[3, 4], Sweilum *et al*. (2005)[3, 4], Tran-Duy *et al*. (2008)[3], Yakubu *et al*. (2013)[3, 4], Yi *et al*. (1996)[3]

**Model 6**

Investigated factor: TGC; number of studies/number of data records: 24/155

Studies mentioned for model 6 in the cell above that are marked with superscript 3

**Model 7**

Investigated factor: TGC; number of studies/number of data records: 14/86

Studies mentioned for model 6 in the cell above that are marked with superscript 4

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CHAPTER 3

# 3

# Genotype by environment interaction between aerated and non-aerated ponds and the impact of aeration on genetic parameters in Nile tilapia (*Oreochromis niloticus*)

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## Abstract

A major problem in smallholder Nile tilapia (*Oreochromis niloticus*) farms is that the achieved production is much lower than under optimal management. One of the main environmental factors contributing to lower production is dissolved oxygen (DO), because the majority of Nile tilapia production takes place under smallholder farms with no aeration of ponds which leads to large DO fluctuations. On the contrary, breeding programs are selecting fish in aerated ponds. Aerating ponds is currently not an option for smallholder farmers because either it is too expensive or they lack access to cheap electricity supply. Therefore, it is crucial to know the genetic correlation between aerated and non-aerated ponds to optimize breeding programs to select fish that perform well in ponds with fluctuating DO levels. The objectives of this study were to 1) investigate the presence of genotype by environment (GxE) interaction between aerated and non-aerated earthen ponds using a design that minimized common environmental effects and 2) the impact of (non-)aeration on genetic parameters. The experimental fish were mass-produced using natural group spawning and nursed in four 30$m^2$ hapas. A random sample of fingerlings from each hapa was tagged and randomly distributed to aerated and non-aerated ponds for a grow-out period of 217 and 218 days. Body weight and photographs were taken on five consecutive time points during grow-out. Of the stocked fish, 2063 were genotyped-by-sequencing. A genomic relationship matrix was built using 11,929 SNPs to estimate genetic parameters with ASReml. No-aeration reduced mean harvest weight (HW), survival and thermal growth coefficient (TGC) compared to aeration. Substantial heritabilities (0.14-0.45) were found for HW, TGC, surface area (SA) and body shape, expressed as ellipticity, and low heritabilities (0.03-0.04) for survival in aerated and non-aerated ponds. In both ponds, the environmental effect common to full sibs was not significant. Genetic coefficients of variation were 20 to 23% lower and heritabilities were 19 to 25% lower in non-aerated pond compared to aerated pond, for HW, TGC and survival. Genetic correlations between ponds for HW, standard length, height, SA and TGC were 0.81, 0.80, 0.74, 0.78 and 0.78, respectively. In summary, some GxE interaction between aerated and non-aerated ponds and we found that no-aeration decreased genetic coefficients of variation and heritabilities compared to aerated ponds. Breeding programs are recommended to use half sib information from non-aerated farms or to set up a reference population for genomic selection in non-aerated environment either on station or in farms.

Keywords: Nile tilapia, genotype by environment interaction, harvest weigh, thermal growth coefficient, survival, dissolved oxygen

## 3.1 Introduction

A major problem in smallholder Nile tilapia (*Oreochromis niloticus*) farms is that the achieved production is much lower than under optimal management. This difference in performance is also called yield gap (Mengistu *et al*., 2019). One of the main environmental factors contributing to yield gap is dissolved oxygen (DO) (Mengistu et al., 2019), because the majority of Nile tilapia production takes place under smallholder farms with no aeration of ponds. No-aeration leads to large daily DO fluctuations. On the contrary, breeding programs, like Genetically Improved Farmed Tilapia (GIFT), are selecting fish that have been growing-out in aerated ponds, which means that there is a difference between the selection environment and the majority of the production environments that do not use aeration. A simple solution seems to be that smallholder farms should aerate their ponds, but for smallholder farms aeration is either too expensive or they lack access to cheap and stable electricity supply (Mengistu *et al*., 2019). Therefore, it is crucial to optimize breeding programs to select fish that perform well in non-aerated ponds with fluctuating DO levels.



The key parameter for optimization of breeding programs in the presence of genotype by environment interaction (GxE) is the genetic correlation between environments (Falconer, 1952; Mulder and Bijma, 2005). The presence of GxE leads to reranking, meaning that the best genotype in one environment is not the best genotype in another environment. In a number of studies in Nile tilapia, genetic correlations between fertilized pond with/without feed supplement, cage culture with feed supplement/commercial pellet feed and rice-fish culture (Bentsen *et al*., 2012), between cage and pond environments (Khaw *et al*., 2012) and between low and high input environments (Trọng *et al*., 2013a) were estimated and found to be lower than unity (Bentsen *et al*., 2012; Khaw *et al*., 2012; Trọng *et al*., 2013a). However, genetic correlation estimates for production traits between aerated and non-aerated ponds are lacking. It is hypothesize that no aeration may hinder fish from expressing their genetic potential and result in lower additive genetic variance compared to aerating ponds and that may also lead to genotype by environment interaction between aerated and non-aerated ponds.

Unbiased estimates of the genetic correlation between environments are crucial to optimize breeding programs, but the classical experiments using pedigree relationships and prolonged separate full sib family rearing are prone to yield biased estimates of the genetic correlation. The key problem is the presence of common environmental effects and properly disentangling those common

environment effects from genetic effects. Nile tilapia families for selective breeding programs are traditionally produced by single pair mating, in a mating ratio of one male to two females (Komen and Trong, 2014). The separate rearing of full sib families until tagging size (3-5g) introduces common environmental effects, which explain 10-20% of the phenotypic variance (Gjerde *et al*., 2012; Thoa *et al*., 2016). Rearing until tagging size could take 2-3 months (Trọng *et al*., 2013a). Full sib families resemble each other, because they share the same common environment and share part of their genes. To get more accurate and unbiased estimates of the genetic parameters, Lozano-Jaramillo *et al*. (2019) recommended 1:5 male to female mating ratios in the presence of common environmental effects and 1:1 ratios when there is no common environmental effect. However, it is practically difficult to attain a 1:5 mating ratio. In addition, prolonged communal rearing programs may increase the genetic correlation of traits in different environments and mask detection of GxE interaction, especially for harvest weigh which is the sum of the communal growth and the growth in the grow-out-period (Dupont-Nivet *et al*., 2010). Therefore, to reduce the presence of common environmental effects and reduce the bias in the estimated genetic correlation, it is crucial to shorten the period of family production and use communal full sib rearing to get more accurate GxE estimates. One solution is to produce fry by natural group mating, e.g. groups of 7 males and 15 females or 12 males and 25 females (Fessehaye *et al*., 2006; Trọng *et al*., 2013b) and later genotype the fish to estimate genetic parameters based on a genomic relationship matrix (GREML, VanRaden, 2008; Goddard *et al*., 2011), so removing the need for separate full sib family rearing until tagging size. Such a design with natural group mating and communal rearing is hypothesized to reduce the contribution of common environmental effects to the phenotypic variance. To test this hypotheses, the main objective of this paper was to investigate whether GxE existed between aerated and non-aerated earthen ponds and the impact of (non-)aeration on genetic parameters. The study was performed by designing a GxE experiment that minimizes common environmental effects in Nile tilapia, based on natural group spawning, and G-BLUP parameter estimates, using GBS.

## 3.2 Material and Methods

### 3.2.1 Family production and nursery

The experiment was carried out in the Aquaculture Extension Centre, Department of Fisheries, Jitra, Kedah State, Malaysia. The fish used in this experiment were mass produced from generation 16 of the GIFT breeding program as follows: the male and female breeders were separately conditioned for two weeks in cages in

an earthen pond (3 x 3 x 1m, mesh size 1 cm) before stocking them in mating hapas. Mating was done in four hapas (each 30m2) in a 500m2 earthen pond, which was aerated by a paddlewheel. Eighteen males and 50 female breeders were stocked for 15 days in each of the mating hapas. In total 72 males and 200 females were used.

On the sixteenth day the breeders were removed, and the fry were kept in the same hapas for nursing for duration of 60 days. Fry were fed commercial feed with 43% crude protein and 5% crude fat at a rate of 10-15% of body weight. The feed was divided into three portions and the fry were fed three times a day.

### 3.2.2 Grow-out and pond management



After 60 days of nursery, the fingerlings from each hapa were transferred into four aerated tanks and conditioned for three days before tagging. Feeding was stopped one day before tagging. The fingerlings from the same nursery hapa were combined in one aerated tank and a random sample of fingerlings was anesthetised using clove oil and individually tagged using PIT (passive integrated transponder) tags. At tagging, each fish was photographed, a 1cm2 fin clip sample collected and PIT tag number and body weight (BW) were recorded. The fin clip samples were preserved in 95% ethanol. The tagging, weight recording, fin clip sample collection and photographing was done in four consecutive days. A random sample of an equal number of individually tagged fingerlings from each nursery hapa was stocked in two earthen ponds. Totally 1570 fish were stocked in each pond with a stocking density of 3 fish/$m^2$.

The size of each of the ponds was 511m2 with a water depth of 1 to 1.2 meter. The only treatment difference between the ponds was use aeration. One of the ponds was aerated using a paddle wheel and blower to create a normoxic environment. The second pond was without aerator to create diurnal dissolved oxygen (DO) fluctuations, which is a typical feature of earthen ponds where green algae are the main source of oxygen (Figure 3.1).

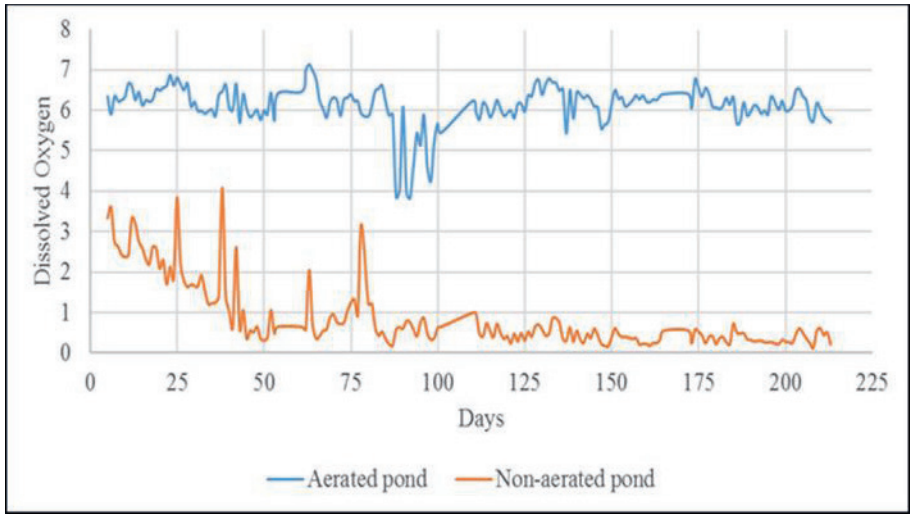


**Figure 3.1** Morning dissolved oxygen in the aerated and the non-aerated pond.

During the grow-out period feeding management was kept the same in both ponds. Commercial feed with 30% crude protein and 5% crude fat at a rate of 5% of body weight per day were used. After 2 months this was reduced to 3% of their body weight per day. The feeding rate was adjusted approximately every three weeks based on a sample of ~100 fish. It was also adjusted based on total biomass and number of fish recorded at each of three time interval measurements (55/56 days, 104/105 days, and 167/168 days). The feed was divided into two portions and fed in the morning from 9:00 to 10:00 and afternoon from 15:00 to 16:00. Some morning feeding was skipped due to cloudy weather conditions that dropped the DO level in a non-aerated pond below 2mg/L. At these concentrations, it was observed that fish no longer eat.

### 3.2.3 Records

Body weight and a photograph of each fish were recorded at stocking, at 55/56 days, 104/105 days, 167/168 days and at harvest, which was after 217 and 218 days of grow-out from the non-aerated pond and aerated pond, respectively. Body weight (g) of fish in each pond was recorded using a digital scale with a precision of one decimal of a gram. Next, each fish was photographed (Olympus OMD EM5 and EM10MKii) together with a metric (cm) ruler and unique labels. Sex was determined at 104/105 days, when the fish was on average 150g. Survival was recorded as the number of days from stocking until the last measurement the fish

was observed alive. In total, fish were measured at stocking, at harvest and three times in between but the focus of this article is on traits at final harvest and survival days.

Standard length (SL) and body height (body depth) (H) measurements of fish were obtained from the picture of each fish taken at stocking and harvest. In total 2063 photographs that were taken at stocking and 1512 photographs that were taken at harvest were loaded into tpsUtil software (Rohlf, 2017b) and digitized for six landmarks using tpsDIG 2.30 (Rohlf, 2017a). The landmarks were as follows: landmarks 1 and 2 were on the 0 and 20cm marks on the ruler which was photographed together with the fish for scaling. The landmarks 3 and 4 were used to measure SL, the distance between the tip of the snout to the base of caudal fin. The landmarks 5 and 6 were the dorsal and ventral landmarks where the distance is maximum. These landmarks were used to calculate H, the maximum dorso-ventral distance (see Figure 3.2 for the landmarks). To get the distance between the Cartesian coordinates, they were analysed in R software using geomorph-package version 3.0.7 (Adams *et al*., 2018) and the true distance was computed based on the reference scale. Photographs for 174 fish at harvest from the non-aerated pond were either missing or the quality was poor. For these fish, the standard length and height were estimated based on linear regression of SL or H on body weight of fish from the same pond: ($Body\ height = 6.41 + 0.007 * body\ weight$ and $Standard\ length = 17.04 + 0.012 * body\ weight$).


**Figure 3.2** Nile tilapia picture with landmarks 1:6. Landmarks 1 and 2 marks a reference scale 20 cm length, landmarks 3 and 4 snout and base of caudal fin, respectively, landmarks 5 and 6 used to measure height (maximum dorso-ventral length) of experimental fish.

Body surface area (SA) of Nile tilapia is similar to the area of an ellipse and was calculated as:

$$SA = \frac{1}{4}\pi * SL * H \quad [1]$$

Ellipticity (Ec) (Blonk et al., 2010) was calculated as:

$$Ec = \frac{(SL-H)}{(SL+H)} \quad [2]$$

Thermal growth coefficient (TGC) (Jobling, 2003) was computed as:

$$TGC = [(\sqrt[3]{W_t} - \sqrt[3]{W_0})/(T \times t)] \times 1000 \quad [3]$$

where $W_t$ is harvest weight, $W_0$ is stocking weight, T is temperature in °C and t is time in days.

The trait survival days was recorded during three interval measurements and at harvest. The trait survival days utilizes the data better than a binomial 0-1 trait for

survived and dead fish, because it accounts for the assumed day of mortality (Ellen *et al*., 2008; Wonmongkol *et al*., 2018).

Dissolved oxygen and water temperature were measured three times a day just before the sunrise, at noon and just before sunset using EcoSense® DO200A. Each time the measurement was taken at three random locations at about two meters from the pond side. Ammonia and pH were measured every week using DR3900 spectrophotometer and EcoTester pH1, respectively.

### 3.2.4 DNA extraction, genotyping, alignment, variant calling and filtering

DNA was isolated from fin clips using the QIAamp DNeasy® 96 Blood and Tissue kit (QIAGEN #69581) following company specifications. DNA yield and quality were checked by full-spectrum spectrophotometer NanoDrop 2000 (Thermo Scientific) and Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). After qualification and quantification, DNA samples were subjected to GBS to identify single nucleotide polymorphisms (SNPs) across the genome.



GBS is a reduced representation approach that uses restriction enzymes to fragment the genome with subsequent size-selection. Individual DNA samples were digested by the ApeKI restriction enzyme and adapters added to both ends of the DNA segments (one end containing a unique barcode to allow pooling of samples in the sequencing process). Fragments of a size between 170 and 350bp were amplified by polymerase chain reaction (PCR) and subsequently sequenced of these libraries on an Illumina HiSeq 2000 sequencing machine.

The cleaned sequence reads were aligned to the Nile tilapia (*Oreochromis niloticus*) reference genome GCF_001858045.1 (Conte *et al*., 2017, https://www.ncbi.nlm.nih.gov/genome/197) by using the BWA-mem algorithm (Version 0.1.75) (Li and Durbin, 2009), for every fish of 2171 individually. Alignments were subsequently re-aligned using GATK IndelRealigner (Van der Auwera *et al*., 2013), and subsequently sorted and indexed by samtools version 0.1.19 (Li *et al*., 2009). For efficient, parallelized, genotype calling using FreeBayes (Garrison and Marth, 2012) the genome was divided in 100kb regions, initially filtered for SNPs to require to have a genotype call rate of at least 70% and a heterozygosity of at least 15%. Variants were further filtered to overlap with expected fragment sizes (in the range 0f 170-350 bp) based on *in silico* prediction of ApeKI restriction sites. Variants of all regions were concatenated and the final

dataset consisted of 42,293 single nucleotide polymorphisms (SNPs). Further stringent quality control was applied using PLINK version 1.9 (Purcell et al., 2007; Purcell, 2018) parameters including the requirement that at least 90% of SNPs were successfully genotyped on all animals, SNP were required to have a minor allele frequency of above 2%, and a genotype call rate for individual fish was required to be at least 70% across all SNPs. The final dataset of 2063 individuals and 11,929 SNPs that passed the quality control thresholds was used for further analyses. The SNPs were distributed throughout the whole genome (Figure 3.3 and 3.4).

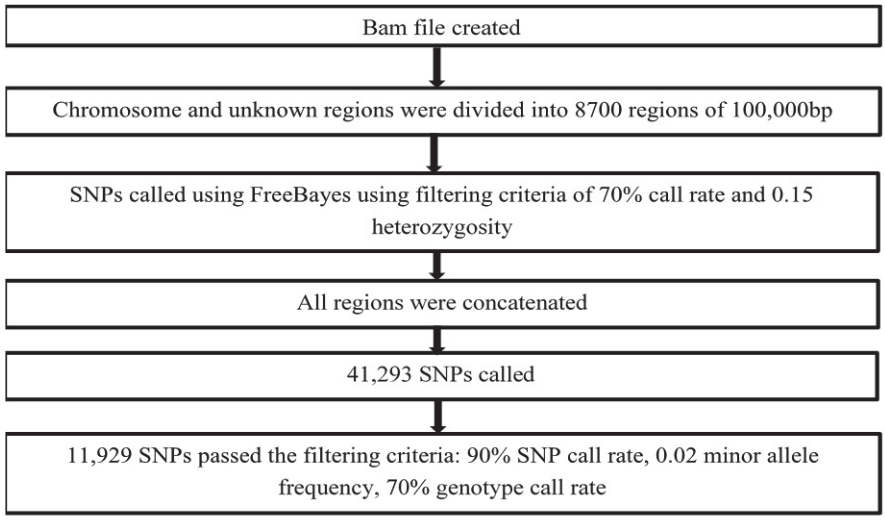


**Figure 3.3** Filtering and quality control flow chart.

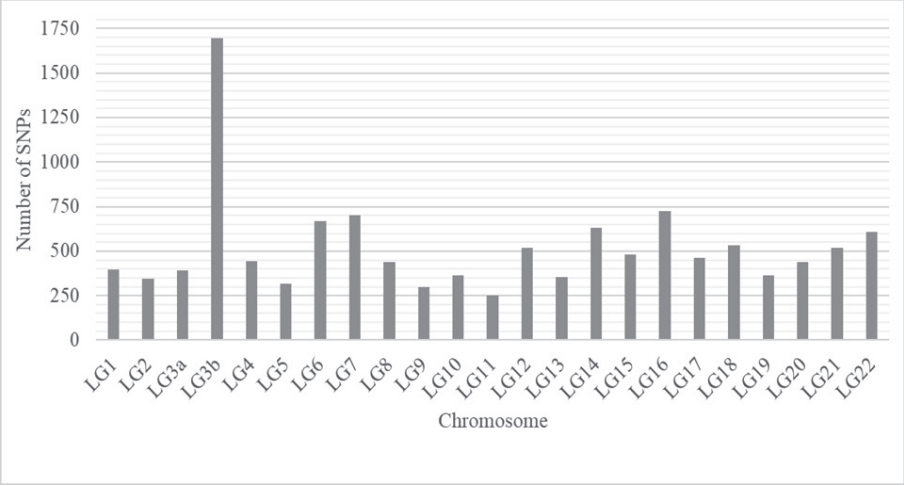




**Figure 3.4** Single nucleotide polymorphisms (SNPs) distribution.

### 3.2.5 Genomic relationship matrix

We computed a genomic relationship matrix (GRM) based on 11,293 SNPs using calc_grm software (Calus and Vandenplas, 2016) using the vanraden2 option. The mean of the diagonal of the computed GRM was 0.83 (GRM1). By definition, the mean of the diagonal of a GRM should be one or higher and furthermore the GRM needs to be invertible. The lower than average diagonal is partly due to the high proportion of missing markers in GBS-data. Therefore, we used two adjusted GRMs:

GRM2: GRM1 was adjusted using the number of non-missing alleles per individual for self-relatedness (diagonal elements) and using the non-missing alleles found on the two individuals for the off-diagonal elements. The adjustment factor $k_{ij}$ for each element of GRM 1 was calculated as:

$$k_{ij} = N_{SNP_{all}}/(N_{SNP_{all}} - N_{SNP_{missing}}) \quad [4]$$

where $N_{SNP\_all}$ was the total number SNP-loci used (11293 SNPs) and $N_{SNP\_missing}$ was the number of SNP-loci missing in a particular individual for diagonal elements of GRM. For off-diagonal elements of the GRM (i.e. the genomic relationship between two animals), and the $N_{SNP\_missing}$ was the number of SNP-loci missing in

at least one of the two individuals; $N_{SNP\_all} - N_{SNP\_missing}$ was therefore the number of SNP-loci with genotype of both individuals.

The element of GRM2 was calculated as:

$$G_{2_{ij}} = k_{ij} G_{ij} \quad [5]$$

GRM3: GRM 2 was multiplied with an extra adjustment factor to make the average of the diagonal elements equal to 1, because the average diagonal element in GRM2 was 0.842. GRM3 was calculated as

$$G_{3_{ij}} = \frac{1}{0.842} G_{2_{ij}} \quad [6]$$

Based on preliminary analysis the genetic parameter estimates from the three GRMs were similar (see Table 3.1 for the variance component estimates for harvest weight using the different GRMs). The analysis presented in this paper is based on GRM3.

**Table 3.1** Additive genetic ($\sigma_a^2$) and residual ($\sigma_e^2$) variances and heritability estimates ($h^2$) and standard error (se) for harvest weight using different genomic relationship matrixes (GRMs) in aerated and non-aerated ponds.

| Pond | GRM | $\sigma_a^2$ | $\sigma_e^2$ | $h^2$ (se) |
|---|---|---|---|---|
| Aerated | GRM1* | 10744.9 | 28525.9 | 0.27 (0.07) |
| | GRM2** | 10013.4 | 28809.4 | 0.26 (0.07) |
| | GRM3*** | 8435.7 | 28809.4 | 0.23 (0.06) |
| Non-aerated | GRM1 | 3367.2 | 12371.8 | 0.21 (0.07) |
| | GRM2 | 3239.0 | 12409.0 | 0.21 (0.07) |
| | GRM3 | 2728.67 | 12409.0 | 0.18 (0.06) |

*GRM1 calculated using calc_grm software using vanraden2 option.

**Calculated using equations 4 and 5.

***Calculated using equation 6.

## 3.2.6 Statistical analysis

### 3.2.6.1 Estimation of phenotypic and genetic parameters within pond

Firstly, variance components and heritabilities for HW, SL, H, SA Ec, TGC and survival days within ponds were estimated using univariate models by residual maximum likelihood (REML), fitting an animal model with a genomic relationship matrix using ASReml version 4.1 (Gilmour et al., 2015). The model used was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} \quad [7]$$

where, $\mathbf{y}$ is the vector of one trait from HW, SL, H, SA, Ec, TGC and survival days, $\mathbf{b}$ is the vector of fixed effects, which were stocking weight, nursery hapas (1-4) and sex (female, male and unknown); $\mathbf{a}$ is the vector of random additive genetic effects with $\mathrm{N} = (\mathbf{0}, \mathbf{G}\sigma_{\mathrm{a}}^2)$ where **G** is the genomic relationship matrix and $\sigma_a^2$ is additive genetic variance, $\mathbf{e}$ is the vector of residual effects with $\mathrm{N} = (0, \mathbf{I}\sigma_{\mathrm{e}}^2)$ where **I** is identity matrix and $\sigma_e^2$ is the residual variance. The $\mathbf{X}$ and $\mathbf{Z}$ are design matrices assigning phenotypic values to the levels of fixed effects and additive genetic effects. Heritability ($h^2$) of each trait was computed as the ratio of additive genetic variance and phenotypic variance($\sigma_p^2$), $h^2 = \frac{\sigma_a^2}{\sigma_p^2}$. For all traits, linear mixed models were used; for the trait survival days a linear mixed model violates the normality assumption, but a linear mixed model has been used before for such a trait and tends to yield similar results as more complex threshold models (Ellen et al., 2008; Wonmongkol et al., 2018).

Secondly, phenotypic and genetic correlations between different traits measured on the same individual within pond were estimated using bivariate linear models. For all the bivariate models the fixed effects were the same as in the univariate models. The additive genetic effects were normally distributed as $N\left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\end{bmatrix}, \mathbf{G} \otimes \begin{bmatrix}\sigma_{a,T1}^2 & r_{a,T12}\sigma_{a,T1}\sigma_{a,T2}\\ r_{a,T21}\sigma_{a,T1}\sigma_{a,T2} & \sigma_{a,T2}^2\end{bmatrix}\right)$ where $\sigma_{\mathrm{a,T1}}^2$ ($\sigma_{\mathrm{a,T2}}^2$) being the additive genetic variance of trait 1 (trait 2) and $r_{a,T12(21)}$ is the additive genetic correlation between trait 1 and 2. The residual effects were normally distributed as $N\left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\end{bmatrix}, \mathbf{I} \otimes \begin{bmatrix}\sigma_{e,T1}^2 & r_{e,T12}\sigma_{e,T1}\sigma_{e,T2}\\ r_{e,T21}\sigma_{e,T1}\sigma_{e,T2} & \sigma_{e,T2}^2\end{bmatrix}\right)$ where $\sigma_{e,T1}^2$ ($\sigma_{e,T2}^2$) is the residual variances for trait 1 (trait 2) and $r_{e,T12(21)}$ is the residual correlation between trait 1 and 2.

### 3.2.7 Estimation of GxE between ponds

To investigate the degree of GxE between aerated and non-aerated ponds, the $r_g$ between the same traits measured on different individuals in the aerated and non-aerated ponds were estimated with a bivariate model. The model used was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} \quad [8]$$

where, $\mathbf{y}$ is the vector of either HW, SL, H, SA, Ec, TGC or survival days measured on different individuals in the aerated and non-aerated ponds. The environmental

covariance was set to zero, because individual fish cannot be tested in two environments at the same time. The fixed effects and the genetic variance-covariance matrix are the same as described above except for the residual variance-covariance matrix (R) $\begin{bmatrix} \sigma^2_{e,Ap} & 0 \\ 0 & \sigma^2_{e,NAp} \end{bmatrix}$ where $\sigma^2_{e,Ap}$ ($\sigma^2_{e,NAp}$) is the residual variances for a trait in aerated pond (non-aerated pond).

The $r_g$ between the same traits were not estimable as the bivariate model did not converge when the whole data set (N=2063) was used. Alternatively, a subset of data based on clustering fish using genomic relationships was used and analysed with GREML (Chu et al., 2019)

Individuals that were poorly linked to others were removed because this could result in fewer fish with a better genetic connectedness and alleviate the model convergence problem. STRUCTURE software version 2.3.4 (Pritchard *et al*., 2000; Falush *et al*., 2003; Falush *et al*., 2007; Hubisz *et al*., 2009) was used to cluster fish in each hapa into 10 groups, creating a total of 40 groups with closer than average relationship. After the probability that each fish belonged to each group (prob.) was obtained, four probability thresholds (≥0.5, ≥0.6, ≥0.65 and ≥0.70) were used to exclude less related individuals from each group. The individuals from each hapa that passed the screening were merged which resulted in 1309, 1012, 827 and 802 fish with a probability of ≥0.5, ≥0.6, ≥0.65 and ≥0.7, respectively. Bivariate analyses using the 1309, 1012, 827 and 802 fish data sets were undertaken. Only the data set with 802 fish that passed the ≥0.7 threshold probability converged and these parameters are reported here.

## 3.3 Results

### 3.3.1 Descriptive statistics

Dissolved oxygen and temperature were recorded daily. The average morning DO and temperature in the aerated pond were 6.0 mg/l and 28.6°C, respectively. The average morning DO and temperature levels in the non-aerated pond were 0.9 mg/l and 27.3°C, respectively. The morning DO in the non-aerated pond decreased over time as fish got bigger and was much lower than the minimum requirement of 3.0 mg/l (Figure 3.1). The average dissolved oxygen around 1:00 pm and 6:00 pm were both above 5 mg/l in both ponds. The average unionized ammonia (UIA) was

0.03 mg/l in both ponds. The average pH was 7.4 and 7.3 in the aerated and the non-aerated pond, respectively.

The number of fish harvested from the aerated pond and non-aerated pond were 1005 and 899, respectively. Survival in the aerated and non-aerated pond was 64.0% and 57.2%, respectively. Survival was significantly ($P < 0.001$) higher in the aerated pond than the non-aerated pond. Feed conversion ratio (FCR) was 1.73 and 2.31 in the aerated and non-aerated pond, respectively (Table 3.2).

**Table 3.2** Total number of stocked and harvested fish, survival percentage and feed conversion ratio (FCR) in aerated and non-aerated pond.

| Pond | Number of fish stocked | Number of fish harvested | Survival (%) | FCR |
|---|---|---|---|---|
| Aerated | 1570 | 1005 | 64.0 | 1.73 |
| Non-aerated | 1572 | 899 | 57.2 | 2.31 |



Descriptive statistics of harvest weight, for each sex and combined, are presented in Table 3.3 and in the Table 3.8 (See Appendix A). The mean weights in the four hapas were different, but the average stocking weights in the aerated (25.4 g) and non-aerated (24.8 g) ponds, were similar. However, the coefficient of variation was somewhat higher in the non-aerated pond (53.9%) compared to the aerated pond (51.8%), due to random sampling effects (Table 3.3). The mean harvest weight was 781.4 g for the aerated pond and 578.5 g for the non-aerated pond. Males were 36.6% and 26.6 % heavier than females at harvest (Table 3.3) in the aerated and non-aerated pond respectively. The coefficient of variation (CV) for harvest weight for females was higher in both aerated (31.1%) and non-aerated ponds (25.0%) than for males in both aerated (22.3%) and non-aerated ponds (19.4%). Survival, expressed as days to (assumed) mortality, was higher in the aerated pond (199.9 +/- 47.6) than in the non-aerated pond (190.2 +/- 53.5; Table 3.3). There was no difference in shape (Ec) between the two ponds (mean value 0.4). In summary, pond aeration lead to a higher mean harvest weight, higher survival and better FCR compared to non-aeration.

**Table 3.3** Number of fish (N), mean body weights (g), mean survival days, coefficient of variation (CV, %) and standard deviation (SD) of genotyped fish per pond.

| Environment | Sex | At stocking | | | | At harvest | | | | Survival days | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | Mean | SD | CV | N | Mean | SD | CV | N | Mean | SD | CV |
| Aerated pond | Male | 484 | 26.6 | 14.2 | 53.1 | 451 | 952.1 | 211.9 | 22.3 | 484 | 212.1 | 23.2 | 10.9 |
| | Female | 459 | 24.1 | 11.8 | 48.9 | 434 | 603.3 | 187.6 | 31.1 | 459 | 212.6 | 23.12 | 10.9 |
| | Not determined | 83 | 26.1 | 13.9 | 53.3 | | | | | 83 | 58 | | |
| | All sex | 1026 | 25.4 | 13.2 | 51.7 | 885 | 781.4 | 265.5 | 34.0 | 1026 | 199.9 | 47.6 | 23.8 |
| Non-aerated pond | Male | 440 | 24.9 | 14.0 | 56.2 | 388 | 669.9 | 129.6 | 19.4 | 440 | 209.1 | 20.7 | 9.9 |
| | Female | 468 | 24.7 | 13.1 | 53.0 | 413 | 492.6 | 123.3 | 25.0 | 468 | 208.8 | 20.7 | 9.9 |
| | Not determined | 129 | 25.1 | 12.5 | 50.0 | | | | | 129 | 58 | | |
| | All sex | 1037 | 24.8 | 13.4 | 543.0 | 801 | 578.5 | 154.34 | 26.7 | 1037 | 190.2 | 53.5 | 28.1 |

### 3.3.2 Phenotypic and genetic parameters estimation within ponds

Estimates of variance components and heritability ($h^2$) from univariate models for HW, SL, H, SA, Ec, TGC and survival days in the aerated and non-aerated ponds are presented in Table 3.4. For all traits, variance estimates were lower in the non-aerated pond. The genetic coefficients of variation in the non-aerated pond were 9.7 to 47.2% lower compared to the aerated pond (Table 3.4). The $h^2$ estimates for HW, H, SA, Ec and TGC in the aerated and non-aerated ponds were moderate to high, ranging from 0.14 to 0.45 with small standard errors (0.05 to 0.07). The common environmental effects to full sibs, which were in our case fixed rearing hapa effects, were not significant in both ponds. All $h^2$ were higher by 4.8 to 65.2% in the aerated pond compared to the non-aerated pond for all the traits, except Ec. The $h^2$ estimates for survival days were low and with large standard errors, 0.04±0.03 and 0.03±0.02 in the aerated and non-aerated ponds, respectively.



**Table 3.4** [Text Additive genetic ($\sigma_a^2$) and residual ($\sigma_e^2$) variances, genetic coefficient of variation (GCV) and heritability ($h^2$) and its standard error (se) estimates from univariate models for harvest weight (HW), standard length (SL), body height (H), surface area (SA), ellipticity (Ec), thermal growth coefficient (TGC) and survival days in aerated (A) and non-aerated (NA) ponds.

| Trait | Pond | $\sigma_a^2$ | $\sigma_e^2$ | GCV | $h^2$ (se) |
|---|---|---|---|---|---|
| HW | A | 8435.72 | 28809.40 | 0.118 | 0.23 (0.06) |
| | NA | 2728.57 | 12409.00 | 0.090 | 0.18 (0.06) |
| SL | A | 0.91 | 2.97 | 0.037 | 0.23 (0.06) |
| | NA | 0.22 | 2.43 | 0.019 | 0.08 (0.05) |
| H | A | 0.25 | 0.92 | 0.045 | 0.21 (0.06) |
| | NA | 0.14 | 0.56 | 0.036 | 0.20 (0.06) |
| SA | A | 288.02 | 1157.20 | 0.072 | 0.20 (0.06) |
| | NA | 106.28 | 672.10 | 0.052 | 0.14 (0.06) |
| Ec | A | 0.00014 | 0.00024 | 0.030 | 0.37 (0.07) |
| | NA | 0.00012 | 0.00015 | 0.027 | 0.45 (0.07) |
| TGC | A | 0.0042 | 0.0121 | 0.064 | 0.26 (0.06) |
| | NA | 0.0019 | 0.0073 | 0.051 | 0.21 (0.06) |
| Survival days | A | 21.93 | 472.38 | 0.110 | 0.04 (0.03) |
| | NA | 12.39 | 376.34 | 0.065 | 0.03 (0.02) |

Phenotypic and genetic correlations are presented in Tables 3.5 (aerated pond) and 6 (non-aerated pond). As expected, the phenotypic ($r_p$) and genetic correlations ($r_g$) between HW and body size traits (SL, H, SA) were high ($r_p$ =0.91 to >0.99, $r_g$ = 0.85 to >0.99). The genetic correlation between HW and SA was close to unity in both ponds ($r_g$ = 0.99 for the non-aerated pond and $r_g$ >0.99 for the aerated pond). The estimated $r_g$ between TGC and HW or SA was 0.97 in the aerated pond, and 0.84 - 0.86 in the non-aerated pond, indicating that SA and HW describe genetically the same trait.

The estimated $r_g$ between Ec and HW were -0.25±0.17 and -0.48±0.17 in the aerated and the non-aerating ponds, respectively. A negative value means that genetically larger fish are rounder (ellipse value closer to zero) than smaller fish. The estimated $r_g$ between Ec and TGC in both ponds were also low and negative (-0.26 to -0.38), indicating that fish that grow fast are more round.

The estimated $r_g$ between survival days and traits such as HW, body size traits (SL, H and SA) and TGC in the aerated pond were low and negative with large standard errors (-0.00 to -0.29, Table 3.5). Taking into account the large standard errors, these genetic correlations suggest that fish that have genetically higher HW, body size and TGC had less survival days than fish with lower HW, body size and TGC. Similarly, the estimated $r_g$ between survival days and SL (-0.50±0.24) and survival and H (-0.14±0.37) in the non-aerated pond were low and negative with large standard errors (Table 3.6), suggesting that survival days reduces genetically with increasing HW and body size. Longer fish has less survival days in the non-aerated pond than the aerated pond. Genetic correlations between survival and Ec in both ponds and between survival and traits such as HW, Ec and TGC in the non-aerated pond could not be estimated due to model convergence problems. Phenotypic correlations between survival and other traits at harvest were not estimated because all fish that survived until harvest had the same survival. Because of lack of variation in survival days in the survived fish, there were no residual correlations. Although the standard errors were large, the negative $r_g$ between survival days and HW, body size traits and TGC suggest that heavier, large and fast-growing fish had a lower survival rate and therefore fewer survival days, especially in the non-aerated pond.

**Table 3.5** Genetic correlations above the diagonal, phenotypic correlations below the diagonal, heritabilities on the diagonal and standard errors in brackets for harvest weight (HW), standard length (SL), height (H), surface area (SA), ellipticity (Ec) and days of survival of fish in aerated pond.

| | HW | SL | H | SA | Ec | TGC | Survival |
|---|---|---|---|---|---|---|---|
| HW | | 0.95 (0.02) | 0.95 (0.02) | .>0.99 | -0.25 (0.17) | 0.97 (0.01) | -0.07 (0.31) |
| SL | 0.92 (0.01) | | 0.79 (0.07) | 0.94 (0.02) | 0.07 (0.18) | 0.92 (0.03) | -0.00 (0.32) |
| H | 0.93 (0.01) | 0.88 (0.01) | | 0.95 (0.02) | -0.54 (0.13) | 0.93 (0.03) | -0.29 (0.35) |
| SA | >0.99 | 0.96 (0.00) | 0.97 (0.00) | | -0.26 (0.17) | 0.97 (0.01) | -0.15 (0.33) |
| Ec | -0.40 (0.04) | -0.17 (0.04) | -0.60 (0.03) | -0.42 (0.03) | | -0.26 (0.16) | * |
| TGC | 0.97 (0.00) | 0.93 (0.01) | 0.93 (0.01) | 0.96 (0.00) | -0.41 (0.04) | | -0.16 (0.29) |
| Survival | ** | ** | ** | ** | ** | ** | |

*The parameters could not be estimated due to model convergence problem.

**Phenotypic correlations could not be estimated due to no residual variance. All fish with harvest weight measurement had the same survival days.

**Table 3.6** Genetic correlations above the diagonal and phenotypic correlations below the diagonal and standard errors in brackets for harvest weight (HW), standard length (SL), height (H), surface area (SA), ellipticity (Ec) and days of survival of fish in non-aerated pond.

| | HW | SL | H | SA | Ec | TGC | Survival |
|---|---|---|---|---|---|---|---|
| HW | | 0.85 (0.08) | 0.92 (0.03) | 0.99 (0.01) | -0.48 (0.16) | 0.86 (0.05) | * |
| SL | 0.91 (0.01) | | 0.61 (0.17) | 0.86 (0.07) | 0.04 (0.26) | 0.73 (0.13) | -0.50 (0.24) |
| H | 0.94 (0.00) | 0.86 (0.01) | | 0.93 (0.03) | -0.79 (0.10) | 0.78 (0.08) | -0.14 (0.37) |
| SA | 0.97 (0.00) | 0.94 (0.00) | 0.97 (0.00) | | -0.52 (0.17) | 0.84 (0.07) | * |
| Ec | -0.38 (0.04) | -0.11 (0.04) | -0.58 (0.03) | -0.38 (0.04) | | -0.38 (0.16) | * |
| TGC | 0.95 (0.00) | 0.87 (0.01) | 0.91 (0.01) | 0.93 (0.01) | -0.38 (0.04) | | * |
| Survival | ** | ** | ** | ** | ** | ** | |

* The parameters could not be estimated due to model convergence problem.

**Phenotypic correlations could not be estimated due to no residual variance. All fish with harvest weight measurement had the same survival days.

### 3.3.3 GxE between ponds

Genetic correlations of HW, TGC, survival, and body size traits between the aerated and the non-aerated ponds are given in Table 3.7. The bivariate model did not converge when the full dataset was used (N = 2063). After clustering and removing all fish with low relationships (giving N = 802) most genetic correlations could be estimated with a bivariate model (Table 3.7). Correlations were generally high, with high standard errors. The correlations for survival and Ec were not estimable as the model did not converge. The genetic correlation between ponds for HW, SL, H, SA and TGC were 0.81±0.30, 0.80±0.27, 0.74±0.33, 0.78±0.34 and 0.78±0.22, respectively, indicating some degree of GxE interaction.

**Table 3.7** [Text Genetic correlation between the aerated and the non-aerated ponds for harvest weight (HW), standard length (SL), height (H), surface area (SA), ellipticity (Ec), thermal growth coefficient (TGC) and survival days.

| Trait | Genetic correlation |
|---|---|
| HW | 0.81±0.30 |
| SL | 0.80±0.27 |
| H | 0.74±0.33 |
| SA | 0.78±0.34 |
| Ec | † |
| TGC | 0.78±0.22 |
| Survival days | † |

† The genetic correlations were not estimable.



## 3.4 Discussion

The objectives of this study were to investigate the presence of GxE between aerated and non-aerated earthen ponds and the impact of (non-)aeration on genetic parameters by designing a GxE experiment that could minimize common environmental effects. GxE may manifest itself as heterogeneity of variances and re-ranking. In the next three sections, the novel aspects of the experimental design, the impact of (non-)aeration and GxE and the implications of our study for genetic improvement programs are discussed.

### 3.4.1 The experimental approach

Novelties in this experiment were mass production of families to minimize common environmental effects, use of genomic relationships based on GBS and use of digital image analysis (DIA) to measure standard length and height. A common problem in GxE studies in tilapia is the estimation of common environmental effects. In our experiment based on mass spawning and genomic relationships there were very small and not significant common environmental effects, which

approach has clear advantages in addition to minimize common environmental effects. In the experiment families were produced in only 15 days using mass spawning which is much shorter than the 2-3 months required by classical family production (Trọng *et al*., 2013a). Another benefit of mass spawning was that it required less labour and infrastructure. The main disadvantage of mass spawning was that it was not possible to have control over the mating and the number of families produced. A small number of sires may have contributed to a large portion of the offspring (Fessehaye *et al*., 2006). However, the use of genomic information allows the estimation of relationships between and within families, which creates additional power for estimation of the genetic parameters (Visscher *et al*., 2014).

In this study, the family production and family group communal nursery time (70 days) was much shorter than the grow-out length (217 to 218 days). In earlier studies that investigated GxE interactions (Eknath *et al*., 2007; Khaw *et al*., 2009b; Trọng *et al*., 2013a; Omasaki *et al*., 2016), genetic correlations have been estimated using pedigree relationships on animals that experienced a prolonged common environment prior to testing. In these studies, the period of grow-out in production environment was less than the period of time in which families were reared separately in circumstances leading to communal environmental effects. Shorter grow-out periods relative to those for family production could lead to higher genetic correlations due to full sibs spending more time together than in different environments, which may mask GxE (Dupont-Nivet *et al*., 2010). Our estimates of genetic correlations were, however, not very different from previous studies estimating GxE between various environments.

Estimation of genetic correlations between the two ponds using a GRM based on SNPs from GBS, was not trivial, because ASReml did not converge when the whole data set (N = 2063) was used. Three issues may have played a role in this convergence problem: 1. limited sample size, 2. missingness and genotyping errors in GBS data and 3. mass spawning and unbalanced family size. The whole data set with approximately 1000 animals per environment is on the low side for estimating GxE (Sae-Lim *et al*., 2010; Lozano-Jaramillo *et al*., 2019). However, genotypic information can largely decrease the standard error on estimated genetic correlations. For instance, when using the equations by Visscher *et al*. (2014), the standard error on the genetic correlation becomes ~0.1 when assuming that the number of independent chromosomal segments is 500, heritability is 0.3 and the true genetic correlation is 0.8. This suggests that the convergence problem may not

be just low sample size. Secondly, missingness and genotyping errors in GBS data could play a role. The coverage of the GBS data may have yielded a low number of informative SNP genotypes in pairs of individuals. For instance, in the extreme case, when using a threshold of 30% of missing SNP per individual, a pair of two individuals each with 30% missing SNP genotypes and with no overlap in the missing SNPs, could have only 40% of the SNPs used to estimate the genetic relationship between these two individuals. This in itself may create extra noise in the genomic relationships between individuals, because for each pair of individuals different SNPs were used to estimate the genomic relationship. Another sign of the limited quality of the GBS data is the fact that the average of the diagonal elements in the G-matrix was below 1, indicating an excessive amount of heterozygosity across loci which is likely due to poor SNP calling on some loci. Similarly, Similarly, Pérez-Enciso (2014) and Dodds *et al*. (2015) reported in simulated and real data distortion of elements of the G-matrix based on low-coverage GBS data. Thirdly, mass spawning may lead to large differences in family size. Fessehaye *et al*. (2006) found that some families were present in small quantities while other families were very abundant. In the study, the parents were not genotyped and parentage assignment was not possible. Therefore, families could not be equalized in terms of numbers of individuals. However, by clustering animals in groups based on high molecular coancestry, individuals were identified that were poorly related with all other individuals based on genomic relationships. This indeed resulted in an analysis that converged. The genetic correlations in the present study were estimated based on 802 fish which is on the lower side of what was considered an optimal design for estimating genetic correlation using stochastic simulation (Lozano-Jaramillo *et al*., 2019). On the other hand, Ødegård and Meuwissen (2012) showed with simulation that with a low number of families and large family size heritabilities can already be estimated quite accurately just based on within-family relationships using genomics, which was supported by analytical work by Hill (2013). For new experiments, precision of estimated genetic correlations can be improved by genotyping parents to construct the pedigree and remove small families, using a SNP array data instead of GBS and increasing the size of the experiment.

Another novelty in this experiment was the use of DIA to calculate SA and Ec in Nile tilapia. The genetic correlation between HW and SA, and TGC and SA were strong and positive, while the genetic correlation between HW and Ec were negative in both ponds and only significantly deviating from zero in the non-aerated pond (t-test) (Lynch and Walsh, 1998). Therefore, HW, TGC and Ec could be improved by

selecting on the correlated SA, which can be automated using DIA and reduces handling stress. Another advantage of automated DIA is that it allows for multiple measurements to be taken over time so that the moment of mortality can be recorded with much greater precision than based on 3 interval measurements and the final harvest as in the current experiment. The use of DIA is time-efficient and can be stored for later use (Blonk *et al*., 2010), moreover, it is less stressful for the fish than the manual method and avoids recording errors.

### 3.4.2 The impact of (non-)aeration and genotype by environment interactions

Aerating the pond had a positive impact on harvest weight, survival and FCR. The mean harvest body weight was 26% higher in the aerated pond than in the non-aerated pond. Similarly, survival of fish was higher in the aerated pond. The FCR in the aerated pond was 1.73 which is high but an acceptable value, while in the non-aerated pond FCR was too high at 2.31 (Craig, 2009). Aerating ponds kept dissolved oxygen level always above 5 mg/l, while in the non-aerated pond the dissolved oxygen dropped to <1 mg/l during the night. Dissolved oxygen is one of the main factors that affect FCR and growth of Nile tilapia (Mengistu *et al*., 2019). Under hypoxia (3 mg/l), Nile tilapia significantly underperform in terms of FCR and growth compared to normoxia (5 mg/l) (Tran *et al*., 2016; Mengistu *et al*., 2019). In summary, these results confirm the findings of Mengistu *et al*. (2019) that aerating ponds results in a higher mean harvest weight, survival, and lower FCR.

In the presence of large environmental differences between selection and production environments, GxE is expected. In the present experiment there were heterogeneity of variances between the aerated and non-aerated ponds and genetic correlations less than unity between ponds. The additive genetic variances and heritabilities for the different traits were lower in the non-aerated pond. For instance, the genetic coefficient of variation for HW, TGC and survival in the non-aerated pond was lower by 23.73%, 20.31% and 40.91%, respectively. Eknath *et al*. (2007) found 46 to 79% lower heritabilities for harvest weight in low input environments compared to high input environment. Non-aeration inhibited the fish from expressing their full genetic potential for growth. This inhibition of growth resulted also in reranking of fish as indicated by the genetic correlations around 0.8 in this study, although the genetic correlation did not significantly deviate from 1 (t - test) (Lynch and Walsh, 1998). To the best of our knowledge, this is the first study publishing genetic correlations between aerated and non-aerated ponds for the traits investigated. However, there have been GxE studies using pedigree

relationships for HW of Nile tilapia between different environments. Our estimate of 0.81±0.30 was similar to those estimates of GxE in other studies: 0.76 - 0.99 between different pond environments (Eknath *et al*., 2007), 0.86 – 0.94 between nucleus, cage and low input pond (Trọng *et al*., 2013a), 0.74 between mixed sex and mono sex Nile tilapia (Omasaki *et al*., 2016), and 0.74 between low input and high input pond environments (Khaw *et al*., 2009a). Our result for TGC 0.78±0.22 was similar with 0.77 between the nucleus breeding environment and low input environment for daily growth coefficient (DGC) reported by Trọng *et al*. (2013a) and higher than the 0.59 between mono-sex and mixed sex Nile tilapia for DGC reported by Omasaki *et al*. (2016). In summary, our study reports for the first time GxE between aerated and non-aerated ponds that results in both heterogeneity of variance and heritability as well as re-ranking of genotypes.



### 3.4.3 Implications for genetic improvement programs

From a genetic improvement perspective, the question is how to select fish in aerated pond that perform better in both aerated and non-aerated ponds. In many pond production environments, farmers usually do not aerate ponds, while the selection environment usually consists of aerated ponds. Therefore, the improvement in performance in a non-aerated pond is a correlated response. A correlated response is less than a direct response when a genetic correlation is less than one, assuming heritabilities are similar in the two environments (Falconer, 1990). Selecting under environments that are not similar with a production environment limits scope of selection of alleles of genes that are responsible for better performance in a production environment (Hammond, 1947). With estimated genetic correlations of about 0.8 in this study and assuming that the true genetic correlation is close to the estimated value, it is clear that the genetic improvement in the nucleus based on aerated ponds will not be fully expressed in production environments without aeration. The advantage of selection in aerated ponds is that the heritability of most traits higher, resulting in a higher accuracy of selection than when selection would be performed in a non-aerated ponds. Nevertheless, if the breeding goal is to increase performance in non-aerated production environments and selection has to be undertaken in an aerated pond, it is advised to use half sib information from on-station non-aerated pond information (Brascamp *et al*., 1985; Mulder and Bijma, 2005).This half sib information reduces the reduction in selection response due to GxE (Brascamp *et al*., 1985; Mulder and Bijma, 2005). However, half sib information requires pedigree data and pedigree data is often lacking in non-aerated ponds in farms. In the experiment, the common environmental effect was successfully reduced by

using natural mating. Genotyping with GBS was however less reliable, probably due to a high rate of genotyping errors. We recommend therefore to collect genomic and phenotypic information from production environments and genotyping with a SNP-chip, to set up reference populations for genomic selection programs to increase response in commercial environments (Mulder, 2016).

## 3.5 Conclusions

Substantial additive genetic variance was found for HW, TGC, survival, body shape and body size measurements in aerated and the non-aerated ponds indicating these traits respond to selective breeding. Mass spawning and use of genomic relationship enabled to minimize common environmental effects to full sibs. Non-aeration led to lower genetic variance and heritabilities. The estimated genetic correlations suggest some GxE for HW, standard length, height, surface area and TGC, although none of the genetic correlations was significantly deviating from unity due to large standard errors. To optimize breeding programs to breed fish that perform well in non-aerated ponds, breeding programs are recommended to use genotypic and phenotypic information from non-aerated on-station ponds to set up a reference population for genomic selection.

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**Appendix A**

**Table 3.8** Descriptive statistics summary for standard length (SL), height (H), surface area (SA), ellipticity (Ec) and thermal growth coefficient (TGC). Number of fish (N), mean, standard deviation (SD) and coefficient of variation (CV, %) of genotyped fish in aerated (A) and non-aerated (N A) ponds.

| Trait | Pond | sex | Measurements at stocking | | | | Measurements at harvest | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | N | Mean | SD | CV | N | Mean | SD | CV |
| SL (cm) | A | Male | 484 | 8.89 | 1.51 | 17.04 | 451 | 27.68 | 1.90 | 6.82 |
| | | Female | 459 | 8.63 | 1.37 | 15.84 | 434 | 24.23 | 2.15 | 8.87 |
| | | Not determined[†] | 83 | 8.80 | 1.59 | 18.06 | | | | |
| | | All sex | 1026 | 8.76 | 1.46 | 16.66 | 885 | 25.99 | 2.67 | 10.23 |
| SL (cm) | NA | Male | 440 | 8.66 | 1.60 | 18.45 | 388 | 25.40 | 1.60 | 6.28 |
| | | Female | 468 | 8.62 | 1.53 | 17.72 | 413 | 23.06 | 1.68 | 7.29 |
| | | Not determined[†] | 129 | 8.74 | 1.35 | 15.48 | | | | |
| | | All sex | 1037 | 8.65 | 1.54 | 17.76 | 801 | 24.19 | 2.01 | 8.32 |
| H (cm) | A | Male | 484 | 3.69 | 0.74 | 20.13 | 451 | 12.59 | 1.08 | 8.54 |
| | | Female | 459 | 3.56 | 0.67 | 18.70 | 434 | 10.29 | 1.20 | 11.61 |
| | | Not determined[†] | 83 | 3.64 | 0.75 | 20.66 | | | | |
| | | All sex | 1026 | 3.63 | 0.71 | 19.63 | 885 | 11.46 | 1.61 | 14.09 |
| H (cm) | NA | Male | 440 | 3.58 | 0.76 | 21.22 | 388 | 11.18 | 0.83 | 7.41 |
| | | Female | 468 | 3.56 | 0.74 | 20.74 | 413 | 9.77 | 0.90 | 9.25 |
| | | Not determined[†] | 129 | 3.62 | 0.65 | 17.96 | | | | |
| | | All sex | 1037 | 3.58 | 0.74 | 20.61 | 801 | 10.45 | 1.12 | 10.69 |
| SA ($cm^2$) | A | Male | 484 | 26.59 | 9.78 | 36.79 | 451 | 275.00 | 39.99 | 14.54 |
| | | Female | 459 | 24.81 | 8.42 | 33.91 | 434 | 197.64 | 39.53 | 20.00 |
| | | Not determined[†] | 83 | 26.09 | 9.75 | 37.38 | | | | |
| | | All sex | 1026 | 25.76 | 9.22 | 35.81 | 885 | 237.07 | 55.47 | 23.40 |

| Trait | Pond | sex | Measurements at stocking | | | | Measurements at harvest | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | N | Mean | SD | CV | N | Mean | SD | CV |
| SA($cm^2$) | NA | Male | 440 | 25.29 | 9.85 | 38.94 | 388 | 223.73 | 28.86 | 12.90 |
| | | Female | 468 | 24.99 | 9.41 | 37.65 | 413 | 177.99 | 29.23 | 16.42 |
| | | Not determined[†] | 129 | 25.48 | 8.54 | 33.53 | | | | |
| | | All sex | 1037 | 25.18 | 9.49 | 37.69 | 801 | 200.15 | 36.96 | 18.47 |
| Ec | A | Male | 484 | 0.42 | 0.02 | 4.76 | 451 | 0.38 | 0.02 | 5.03 |
| | | Female | 459 | 0.42 | 0.02 | 4.60 | 434 | 0.40 | 0.02 | 5.19 |
| | | Not determined[†] | 83 | 0.42 | 0.02 | 4.37 | | | | |
| | | All sex | 1028 | 0.42 | 0.02 | 4.67 | 885 | 0.39 | 0.02 | 6.36 |
| Ec | N | Male | 440 | 0.42 | 0.02 | 4.49 | 388 | 0.39 | 0.02 | 4.32 |
| | | Female | 468 | 0.42 | 0.02 | 4.81 | 413 | 0.41 | 0.02 | 4.07 |
| | | Not determined[†] | 129 | 0.42 | 0.02 | 4.56 | | | | |
| | | All sex | 1035 | 0.42 | 0.02 | 4.64 | 801 | 0.40 | 0.02 | 4.67 |
| TGC | A | Male | | | | | 451 | 1.11 | 0.13 | 11.98 |
| | | Female | | | | | 434 | 0.90 | 0.14 | 15.40 |
| | | All sex | | | | | 885 | 1.01 | 0.17 | 17.20 |
| TGC | NA | Male | | | | | 388 | 0.92 | 0.12 | 12.76 |
| | | Female | | | | | 413 | 0.78 | 0.10 | 13.33 |
| | | All sex | | | | | 801 | 0.85 | 0.13 | 15.42 |

| Trait | Pond | sex | Measurements at stocking | | | | Measurements at harvest | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | N | Mean | SD | CV | N | Mean | SD | CV |
| Survival days | A | Male | | | | | 484 | 212.12 | 23.19 | 10.93 |
| | | Female | | | | | 459 | 212.64 | 23.12 | 10.87 |
| | | Not determined† | | | | | 83 | 58.00 | | |
| | | All sex | | | | | 1026 | 199.89 | 47.60 | 23.81 |
| Survival days | N | Male | | | | | 440 | 209.08 | 20.68 | 9.89 |
| | | Female | | | | | 468 | 208.77 | 20.74 | 9.93 |
| | | Not determined† | | | | | 129 | 58.00 | | |
| | | All sex | | | | | 1037 | 190.15 | 53.46 | 28.12 |

† fish which died before sex determination

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CHAPTER 4

# Fluctuations in growth are heritable and apotential indicator of resilience in Nile tilapia (*Oreochromis niloticus*)

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## Abstract

Resilience can be defined as the capacity of an animal to be minimally affected by perturbations or to quickly recover to the state it had before the perturbation. When applied to production animals, resilience is defined as consistency in production over time. This consistency can be quantified by the variance of deviations from the expected trait level measured at multiple time-points. The objectives of this study were to estimate genetic parameters for resilience in Nile tilapia, measured as consistency in growth. We used log-transformed variance of deviations (LnVar) of body weight measured five times during grow-out in either an aerated or a non-aerated pond. The hypothesis was that fish grown in non-aerated ponds are more challenged by environmental conditions, such that heritable variation in LnVar of body weight is more expressed showing larger differences between more and less resilient fish. The heritability for LnVar was 0.10 in aerated pond and 0.12 in the non-aerated pond. In aerated ponds the genetic correlation ($r_g$) of LnVar with harvest weight (HW) was 0.36±0.26, and with thermal growth coefficient (TGC) it was 0.47±0.21. In the non-aerated pond, the $r_g$ with HW and TGC were close to zero (-0.01±0.29 and -0.08±0.22). The genetic correlation for LnVar between both environments was 0.80. These estimates suggest that selection for HW or TGC in aerated ponds will increase LnVar in both environments. Increased LnVar may decrease resilience and this will be detrimental to performance. Selecting for more resilient fish would lead to more constant growth rates, which makes biomass estimation more accurate and could therefore result in more optimal feeding regimes and less feed waste. This would have a favourable effect on the feed efficiency in production units and on the environmental impact of fish farming. To improve resilience together with growth we recommend that fish breeding programs collect repeated records on body weight, preferably in challenging environments.

Keyword: heterogeneity of variance, uniformity, resilience, Nile tilapia, genetic improvement.

## 4.1 Introduction

Resilience can be defined as the capacity of an animal to be minimally affected by perturbations or to quickly recover to the state it had before the perturbation (Colditz and Hine, 2016) . When applied to production animals, resilience can also be defined as consistency of production over time. Resilience indicators are then based on all production deviations due to unknown disturbances during a production cycle (Scheffer *et al*., 2018; Berghof *et al*., 2019b). In animal production, there can be many perturbing factors, for example competition for feed, physical or environmental stressors, disease pressure, and hypoxia (in aquaculture). It is assumed that animals that show consistency in their production are less affected by these perturbation factors compared to animals that show less consistency in their production (Berghof *et al*., 2019a).

The genetic parameters of resilience can be analyzed from repeated or longitudinal records of a trait over time per individual (Friggens *et al*., 2017). Possible resilience indicators that can be calculated from these records are variance of deviations from the mean, autocorrelations between measurements, skewness of deviations or a slope of reaction norm (Berghof *et al*., 2019b). Only a few studies investigated resilience over time, using repeated records (summarized in Table 4.1, see also review by Iung *et al*. (2020)). Of these, natural-log-transformed variance of deviations from the mean (LnVar) appears to be the most promising, given the observed heritability's (range: 0.10-0.24) and GCV's (range: 0.23-0.34). LnVar can easily be calculated from longitudinal records on body weight that represent growth. More resilient animals are expected to show lower values for LnVar compared to less resilient animals. In chicken, (Berghof *et al*., 2019a) found a heritability of 0.10 and a substantial genetic coefficient of variation (0.30) for LnVar based on seven body weight records, measured every 4 weeks.

There are no known estimates of LnVar in aquaculture species. In this study, we investigated the genetic parameters of LnVar for growth in Nile tilapia (*Oreochromis niloticus*). Nile tilapia is the most widely cultured tropical fish species, with annual production exceeding 4.5 million tons globally in 2018 (FAO, 2020). Tilapia are cultured in a wide range of environments ranging from ponds to cages, and several strains have been developed and selected for increased growth rate for more than 15 generations (i.e. GIFT, Bentsen *et al*., 2017). This makes the study of resilience for growth based on repeated body weight measurements of particular interest for Nile tilapia.

In addition to obtaining repeated records per animal over time, it is important to choose the environment under which resilience is investigated. In Nile tilapia, oxygen availability is one of the major factors determining growth, health and survival, especially in non-aerated ponds (Mengistu *et al*., 2020b). Under optimal conditions (i.e. in aerated ponds) Nile tilapia are able to fully express their genetic potential for growth. However, without aeration, ponds show diurnal hypoxia which creates a challenging environment (Mengistu *et al*., 2020a). In such challenging environments more resilient tilapia may grow better and show better survival. We hypothesize that less resilient fish grown in non-aerated ponds are more challenged by environmental conditions and will show higher log-transformed variances of deviances (LnVar) for body weight. Therefore, the objectives of this study were: i) to estimate genetic parameters of resilience using LnVar of body weight measured at five times points in aerated and non-aerated ponds, and ii) to estimate genetic correlations ($r_g$) between resilience and growth rate in both environments.

**Table 1** Summary of studies that investigated resilience based on repeated trait measurements.

| Species | Measurement Method | heritability | GCV | Remark | References |
|---|---|---|---|---|---|
| Dairy cattle | Absolute change in daily milk yield (dMY), residual absolute change in dMY and standard deviation in DMY | 0.10 to 0.20 | - | 5 to 200 days in milk records were used | Moncur *et al.* (2021) |
| Dairy cattle | Log-transformed variances of deviations (LnVar) of three periods of the first lactation and the first three full lactations | 0.12 to 0.20 | - | Data and data preparation were the same as Poppe *et al.* (2020) | (Poppe *et al.*, 2021) |
| | Lag-1 autocorrelation of deviations from lactation curve | 0.05 to 0.08 | - | | |
| | Average daily milk yield | 0.32 to 0.45 | - | | |
| Sheep | Natural logarithm of daily coefficient of variation of daily feed intake (DFI) | 0.08 to 0.14 | - | 951 lambs tested, 51832 DFI records | (Garcia-Baccino *et al.*, 2021) |
| Dairy cattle | Milk yield | 0.20 to 0.24 | 0.23 to 0.25 | Cows with at least 95% daily milk yield records were included, | Poppe *et al.* (2020) |
| | Log-transformed variances based on quantile regression curve | | | | |
| | autocorrelation | 0.08 to 0.10 | 0.07 to 0.17 | The first 350 days milk record were included | |
| | Milk yield, skewedness of deviations from lactation curve | 0.01 to 0.02 | 0.05 to 0.10 | 330 to 350 daily milk yield records per individual | |

| Species | Measurement Method | heritability | GCV | Remark | References |
|---|---|---|---|---|---|
| Pigs | LnVar of litter size in different parities | 0.02 | 0.16-0.17 | | Dobrzański *et al*. (2020) |
| Pigs | Root mean square error of prediction (RMSEP) of daily feed intake and feed duration | 0.15 to 0.26 | - | RMSEP is closely related to the variance of deviations | Putz *et al*. (2019) |
| Chicken | Log-transformed variances of body weight | 0.10 | 0.30 | Seven body weight records over time per individual | Berghof *et al*. (2019a) |
| | Skewness of deviations within an individual | 0.09 | 1.56 | | |
| | Lag-one autocorrelation of deviations within an individual | 0.11 | 0.52 | | |
| Dairy cattle | Milk yield Log-transformed variance within cow | 0.10 | - | Cows with at least 21 consecutive daily milk yield records, and up to 355 days record were included | Elgersma *et al*. (2018) |
| Nile tilapia (*Oreochromis niloticus*) | Log-transformed variances | 0.10 to 0.12 | 0.30 to 0.34 | Five body weight records per individual over time | This study |

## 4.2 Material and Methods

The production, raising, and harvesting of fish is described in detail in (Mengistu *et al*., 2020a), and is summarized below.

### 4.2.1 Family production and nursery

The experiment was carried out in the Aquaculture Extension Centre, Department of Fisheries, Jitra, Kedah State, Malaysia. The fish used in this experiment were mass produced from generation 15 of the GIFT breeding program as follows: The male and female breeders were conditioned for two weeks separately in cages in an earthen pond before stocking them in mating hapas. Mating was done in four hapas (each 30$m^2$) in a 500 $m^2$ earthen pond, which was aerated by a paddlewheel. Eighteen males and 50 female breeders were stocked for 15 days in each of the mating hapas. In total 72 males and 200 females were used.

On the sixteenth day the breeders were removed, and the fry were kept in the same hapas for nursing for a duration of 60 days. The fry were fed commercial feed with 43% crude protein and 5% crude fat at a daily rate of 10-15% of body weight. The feed was divided into three portions and the fry were fed three times a day.



### 4.2.2 Grow-out and pond management

After 60 days of nursery, the fingerlings from the same hapa were transferred into one of four aerated tanks and conditioned for three days before tagging. Feeding was stopped one day before tagging. From each tank a random sample of fingerlings was anesthetised using clove oil and individually tagged using Passive Integrated Transponder (PIT) tags. At tagging, a 1 $cm^2$ fin clip sample was collected and PIT tag number and body weight (BW) were recorded. The fin clip samples were preserved in 95% ethanol. The tagging, weight recording, fin clip sample collection and photographing was done in four consecutive days. A random sample of an equal number of individually tagged fingerlings from each nursery hapa was stocked in two earthen ponds. Totally 1570 fish were stocked in each pond with a stocking density of 3 fish/$m^2$

The size of each of the ponds was 511$m^2$ with a water depth of 1.0 to 1.2 meters. One of the ponds was aerated using a paddle wheel aerator and air blower to create a normoxic environment. The second pond was without aerator resulting in diurnal dissolved oxygen (DO) fluctuations.

During the grow-out period fish were initially fed commercial feed with 30% crude protein and 5% crude fat at a daily rate of 5% of body weight. After 2 months feeding rate was reduced to 3% of body weight. The feeding rate was adjusted approximately every three weeks based on the weight of a sample of ~100 fish. It was also adjusted based on total biomass and number of fish recorded at each time point when body weight was recorded. The feed was divided into two portions and fed in the morning from 9:00 to 10:00 and afternoon from 15:00 to 16:00. Some mornings feeding was skipped due to cloudy weather conditions that made the DO level in the non-aerated pond drop to below 2 mg/L. At these concentrations, it was observed that fish no longer fed.

### 4.2.3 Records

Body weight of each fish was recorded, using a digital scale, at stocking, at 55/56 days, 104/105 days, 167/168 days after stocking, and at harvest, which was after 217 and 218 days of grow-out in the non-aerated pond and aerated pond, respectively. Thermal growth coefficient (TGC) (Jobling, 2003) was computed as:

$$TGC = [(\sqrt[3]{(W_t)} - \sqrt[3]{(W_0)}) / (T \times t)] \times 1000 \quad [1]$$

where $W_t$ is harvest weight, $W_0$ is stocking weight, T is temperature in °C and t is time in days.

### 4.2.4 Genomic relationship matrix

DNA extraction and genotyping are described in Mengistu *et al*. (2020a). In total, records from 1686 genotyped fish were available for the analyses. We computed a genomic relationship matrix (GRM) based on 11,293 SNPs using calc_grm program (Calus and Vandenplas, 2016) using the vanraden2 option. The resulting GRM was adjusted using the number of non-missing alleles per individual for self-relatedness (diagonal elements) and using the non-missing alleles found on the two individuals for the off-diagonal elements, as follows:

$$k_{ij} = \frac{N_{SNP_{all}}}{N_{SNP_{all}} - N_{SNP_{missing}}} \quad [2]$$

And

$$G_{2_{ij}} = k_{ij} G_{ij} \quad [3]$$

where $N_{SNP_{all}}$ is the total number SNP-loci used (11,293 SNPs) and $N_{SNP_{missing}}$ is the number of SNP-loci missing in a particular individual for diagonal elements of GRM. For off-diagonal elements of the GRM (i.e. the genomic relationship between two animals), $N_{SNP\_missing}$ was the number of SNP loci missing in at least one of the two individuals; $N_{SNP\_} - N_{SNP\_\text{missing}}$ was therefore the number of SNP-loci with genotypes for both individuals. Finally, $G_2$ was multiplied with an extra adjustment factor to make the average of the diagonal elements equal to 1 (Mengistu *et al*., 2020a):

$$G_{3_{ij}} = \frac{1}{0.842} G_{2_{ij}} \quad [4]$$

### 4.2.5 Calculation of log-transformed variance of the standardized deviation: LnVar

First, mean body weight ($\overline{Wt}$) and standard deviation (SD) of body weight for the fish belonging to the same nursery hapa, sex and grow-out pond (cohort) was calculated, for each measurement *t* separately. Standardized deviations of body weight were calculated as:

($Wt, i - \overline{Wt, i}$ ) / SD, with *t* the measurement number (1-5), and *i* = cohort.

Next, for each fish, the mean and variance of the resulting 5 standardized deviations was calculated (Berghof *et al*., 2019a). Finally, this variance ("Var-dev") was log-transformed using the natural logarithm to obtain LnVar, which is the commonly used scale to express genetic variation in environmental/residual variance or uniformity in other studies (Hill and Mulder, 2010; Iung *et al*., 2020).

### 4.2.6 Genetic parameter estimation

Phenotypic and genetic variances were estimated using ASReml version 4.1 (Gilmour *et al*., 2015) fitting an animal model with a genomic relationship matrix. Phenotypic ($r_p$) and genetic ($r_g$) correlations between LnVar and, harvest weight (HW) and TGC within aerated and non-aerated ponds; and $r_g$ for LnVar between the aerated and non-aerated ponds were estimated from fitting bivariate linear models. The linear mixed models were:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} \quad [5]$$

where, $\mathbf{y}$ is the vector of one of the traits LnVar, HW or TGC for the univariate models or two of those traits for the bivariate models, $\mathbf{b}$ is the vector of fixed

effects which were nursery hapa (1-4), sex (female, male, and not determined) and stocking weight (fitted only for HW), $\mathbf{a}$ is the vector of random additive genetic effects, $\mathbf{e}$ is the vector of residual effects. The $\mathbf{X}$ and $\mathbf{Z}$ are design matrices assigning phenotypic values to the levels of fixed effects and additive genetic effects respectively. The additive genetic effects were normally distributed as $\mathrm{N}\left([\mathbf{0}],\ \mathbf{A} \otimes \begin{bmatrix} \sigma^2_{a,T1} & r_{a,T12}\sigma_{a,T1}\sigma_{a,T2} \\ r_{a,T12}\sigma_{a,T1}\sigma_{a,T2} & \sigma^2_{a,T2} \end{bmatrix}\right)$ with $\sigma^2_{\mathrm{a,T1}}$ ($\sigma^2_{\mathrm{a,T2}}$) being the additive genetic variance of trait 1 (trait 2) and $r_{a,T12}$ the additive genetic correlation between trait 1 and 2.

Heritability of each trait was computed as the ratio of genetic variance and phenotypic variance, $h^2 = \frac{\sigma^2_a}{\sigma^2_p}$, where $h^2$ is heritability, $\sigma^2_a$ is additive genetic variance and $\sigma^2_p$ is phenotypic variance. The approximate standard errors (SE) were derived from the average information matrix (Fischer *et al*., 2004). The 95% confidence interval for the heritabilities were calculated as $h^2 \pm 1.96 * SE$. The significance of the genetic correlations were tested using loglikelihood ratio test with one degree of freedom (Lynch and Walsh, 1997) comparing a model without constraining the covariance against a model where the covariance was constrained to zero. Genetic coefficient of variation (GCV) for LnVar was calculated as: $GCV = \sqrt{\sigma^2_{a-LnVar}}$ because the log transformation implicitly assumes an exponential model which makes $\sigma^2_{a-LnVar}$ unitless (Mulder *et al*., 2007). For the other traits GCV was calculated as: $GCV = \sqrt{\sigma^2_a}/\mu$, where $\mu$ is the phenotypic mean of the population (Hill and Mulder, 2010). The residual effects were normally distributed as $N(\mathbf{0}, \mathbf{I}\boldsymbol{\sigma}^2_{\boldsymbol{e}})$ for the univariate models. For the bivariate models between traits within ponds, the residual effects were distributed as $\mathrm{N}\left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\end{bmatrix}, \mathbf{I} \otimes \begin{bmatrix} \sigma^2_{e,T1} & r_{e,T12}\sigma_{e,T1}\sigma_{e,T2} \\ r_{e,T12}\sigma_{e,T1}\sigma_{e,T2} & \sigma^2_{e,T2} \end{bmatrix}\right)$ where $\sigma^2_{e,T1}$ ($\sigma^2_{e,T2}$) is the residual variance for trait 1 (trait 2) and $r_{e,T12}$ is the residual correlation between trait 1 and trait 2. For bivariate models between traits in different ponds, the residual effects were distributed as $N = \left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\end{bmatrix}, I \otimes \begin{bmatrix} \sigma^2_{e,T1} & 0 \\ 0 & \sigma^2_{e,T2} \end{bmatrix}\right)$. The residual correlations between traits in different ponds were set to zero because each individual was reared in

either an aerated pond or a non-aerated pond and therefore the residual correlation is non-existing.

## 4.3 Results

### 4.3.1 Descriptive statistics

Average body weights at each of the five body weight measurements are presented in Table 4.2 and Figure 4.1. The average body weight was similar at stocking but started to diverge after 55 and 56 days of grow-out in non-aerated and aerated ponds, respectively. Figure 4.2 shows body weight and standardized body weight at each of the five time points for the ten fish with the lowest and ten fish with the highest estimated breeding values (EBV) for LnVar in aerated ponds. Fish with the lowest EBV for LnVar showed more consistency of growth and more consistent standardized body weight at the five time points compared to fish with high EBV for LnVar. A similar, but more extreme, pattern was seen for fish from the non-aerated pond (Figure 4.3). Mean values for LnVar were similar for both ponds but the range in LnVar values was larger in non-aerated pond compared to aerated pond (Table 4.3).



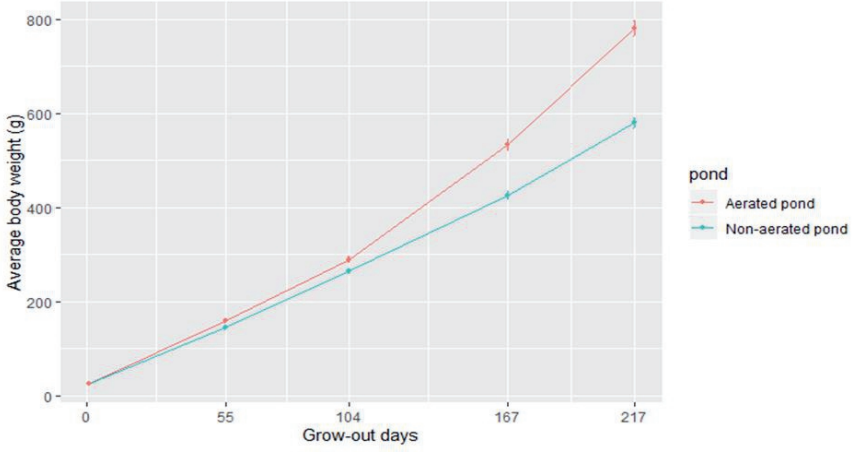


Figure 4.1 Mean body weight with 95% confidence interval at stocking, three interval measurements and at harvest.

Table 4.2 Descriptive statistics of body weight at stocking, at three interval measurements and at harvest, and thermal growth coefficient (TGC)* in aerated and non-aerated ponds.

| | Aerated | | | Non-aerated | | |
|---|---|---|---|---|---|---|
| | N | Mean (sd) | Range | N | Mean (sd) | Range |
| Stocking | 1026 | 25.4 (13.2) | 2.9 – 77.1 | 1037 | 24.8 (13.2) | 3.6 – 77.0 |
| 1 | 1026 | 159.0 (63.2) | 30.2 – 394.3 | 1037 | 144.3 (54.7) | 26 – 328.0 |
| 2 | 941 | 289.2 (92.7) | 63.3 – 650.5 | 907 | 266.1 (73.2) | 70.5 – 498.3 |
| 3 | 903 | 533.4 (177.4) | 68.2 – 1079.1 | 887 | 426.4 (118.8) | 117.0 – 805.0 |
| Harvest | 885 | 781.0 (256.6) | 185.7 – 1588.6 | 801 | 579.9 (154.6) | 135.5 – 1003.4 |
| TGC | 885 | 1.01 (0.17) | 0.47 – 1.46 | 801 | 0.85 (0.13) | 0.41 – 1.18 |

* TGC = $[(\sqrt[3]{(W_t)} - \sqrt[3]{(W_0)}) / (T \times t)] \times 1000$, where $W_t$ is harvest weight, $W_0$ is stocking weight, T is temperature in °C and t is time in days

Table 4.3 Descriptive statistics of variance of deviances (Var-dev), log transformed variance (LnVar), and thermal growth coefficient (TGC) in aerated and non-aerated ponds.

| Trait* | Aerated pond | | | Non-Aerated pond | | |
|---|---|---|---|---|---|---|
| | Mean (sd) | Min | Max | Mean (sd) | min | max |
| Var-dev | 0.5 (0.47) | 0.00 | 3.84 | 0.47 (0.56) | 0.01 | 8.84 |
| LnVar | -1.09 (0.98) | -5.68 | 1.35 | -1.23 (1.03) | -5.27 | 2.18 |
| TGC | 1.01 (0.17) | 0.47 | 1.46 | 0.85 (0.13) | 0.41 | 1.18 |

Trait values were calculated for 885 fish from the aerated pond and 801 fish from the non-aerated pond that had five individual body weight records.

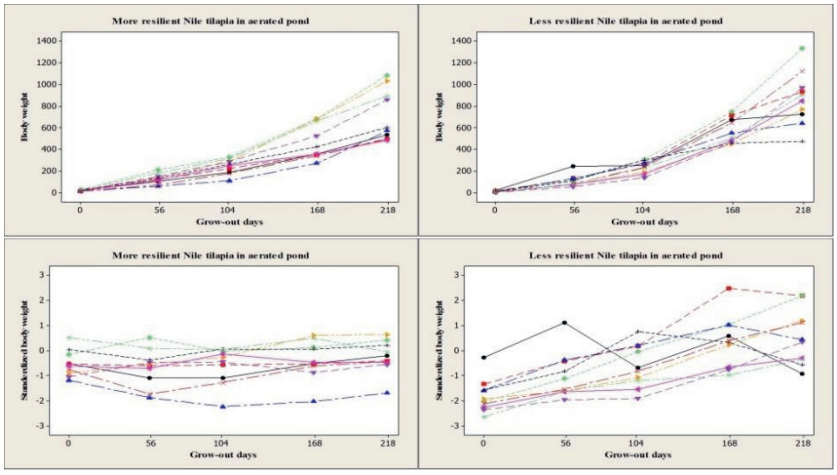


Figure 4.2 Body weight and standardized body weight of ten most resilient and ten least resilient fish from aerated pond, based on genomic estimated breeding values (GEBV) for log-transformed variances (LnVar). Top panels: body weight of ten most resilient fish (left) and ten least resilient fish (right). Bottom panels: standardized weight of ten most resilient fish (left) and ten least resilient fish (right).



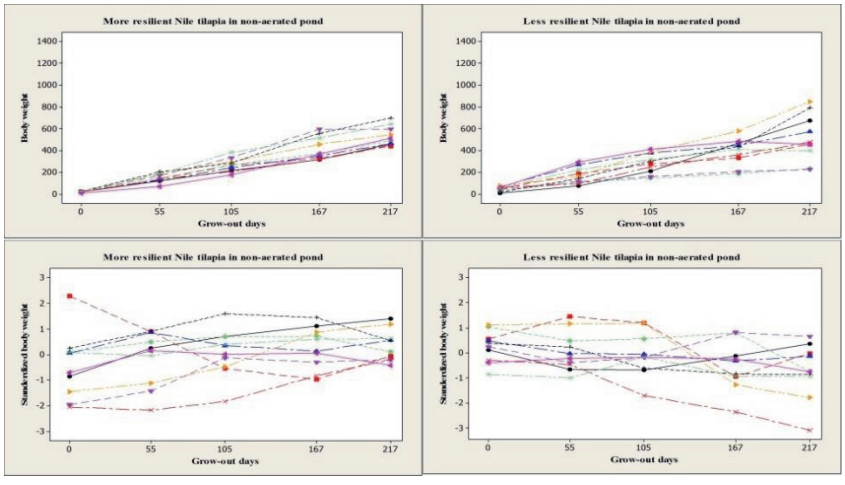


Figure 4.3 Body weight and standardized body weight of ten most resilient and ten least resilient fish from non-aerated pond, based on genomic estimated breeding values (GEBV) for log-transformed variances (LnVar). Top panels: body weight of ten most resilient fish (left) and ten least resilient fish (right). Bottom panels: standardized weight of ten most resilient fish (left) and ten least resilient fish (right).

### 4.3.2 Genetic and phenotypic parameters within ponds

Variances and heritability's from univariate models for LnVar, HW and TGC are presented in Table 4.4. The additive genetic variance for LnVar was substantial in both the aerated and non-aerated pond. The heritability estimate for LnVar was slightly higher in non-aerated pond (0.12±0.04) compared to in the aerated pond (0.10±0.05). The heritability estimates in the aerated pond and in the non-aerated pond were significantly different from zero ($P < 0.05$). However, these heritabilities were not significantly different from each other with 95% confidence interval in the non-aerated pond of [0.022, 0.218] and in the aerated pond of [0.002, 0.198]. The coefficient of variation for LnVar was higher in non-aerated pond than the aerated pond. Contrary to the trend observed for LnVar, the GCVs for both HW and TGC were higher in aerated than in non-aerated ponds. Both the genetic and phenotypic variances for HW and TGC were higher in aerated ponds.

Table 4.4 Additive genetic ($\sigma^2_a$) and phenotypic ($\sigma^2_p$) variances*, genetic coefficient of variation (GCV) and heritability ($h^2$) of log transformed variance based (LnVar), harvest weight (HW) and thermal growth coefficient (TGC) within aerated and non-aerated pond.

| Trait | Aerated | | | | Non-aerated | | | |
|---|---|---|---|---|---|---|---|---|
| | $\sigma^2_a$ | $\sigma^2_p$ | $h^2$±se | GCV | $\sigma^2_a$ | $\sigma^2_p$ | $h^2$±se | GCV |
| LnVar | 0.091 | 0.907 | 0.10±0.05 | 30.2 | 0.118 | 0.988 | 0.12±0.05 | 34.4 |
| HW | 8444.79 | 37274 | 0.23±0.06 | 11.8 | 2791.11 | 15148 | 0.18±0.06 | 9.1 |
| TGC | 0.004 | 0.016 | 0.26±0.06 | 6.3 | 0.002 | 0.009 | 0.21±0.06 | 5.3 |

* Results are from univariate model

### 4.3.3 Genetic correlation

In the aerated pond we estimated a moderate and positive genetic correlation between LnVar and HW (0.36±0.26) and between LnVar and TGC (0.47±0.21) (Table 4.5). In the non-aerated pond however, close to zero genetic correlations between LnVar and HW (-0.01±0.29) and between LnVar and TGC (-0.08±0.22) were estimated. The genetic correlation estimates were not significantly different from zero ($P > 0.05$) except for the genetic correlation between LnVar and TGC in aerated pond ($P < 0.05$). The genetic correlation between environments for LnVar was 0.80±0.17. These results show that LnVar is genetically similar in both environments with limited GxE.

Table 4.5 Genetic and phenotypic correlation between log transformed variance (LnVar) and harvest weight (HW) and thermal growth coefficient (TGC) in aerated pond (A) and non-aerated pond (NA).

| LnVar | Genetic correlation ($r_g$) | | Phenotypic correlation ($r_p$) | |
|---|---|---|---|---|
| with - | A | NA | A | NA |
| HW | 0.36±0.26 | -0.01±0.29 | 0.10±0.04 | 0.04±0.04 |
| TGC | 0.47±0.21 | -0.08±0.22 | 0.09±0.04 | -0.01±0.04 |

## 4 Discussion

In this study, our heritability estimates for LnVar in a non-aerated pond (0.12±0.05) and in an aerated pond (0.10±0.05) were not significantly different from zero and not significantly different from each other. Our heritability estimates for LnVar were considerably higher than heritability estimates reported for uniformity (Table 4.6). These estimates for LnVar are in line with other heritability estimates based on multiple records per individual (Table 4.1). Berghof *et al*. (2019a) also used body weight records, seven per individual chicken, and estimated LnVar heritability at 0.10.



The high genetic correlation (0.8) between LnVar in both environments was significantly different from zero and not different from 1.0, suggesting it is roughly the same trait in both environments. Nevertheless, the GCV for LnVar in non-aerated pond (Table 4.4) may indicate that the genetic variation in LnVar is more expressed in the more challenging non-aerated pond. This higher expression in the non-aerated pond is in contrast with production traits, where challenging environments are expected to suppress the expression of the genetic potential. We did indeed observe lower lower GCVs for production traits HW and TGC in the non-aerated pond compared to the aerated pond. With the genetic correlation of 0.80 for LnVar between aerated and non-aerated ponds, a response in LnVar in non-aerated production environments is possible from data collected in aerated ponds, e.g. in a nucleus breeding station. However given the higher GCV for LnVar in the non-aerated pond the use of sib testing with genomic selection could further increase selection response for LnVar (Mulder, 2016).

The genetic correlations between LnVar and HW (-0.01), and between LnVar and TGC (-0.08) in non-aerated pond and between LnVar and HW (0.36) were not significantly different from zero. The genetic correlations between LnVar and TGC (0.47) in aerated pond were moderate and significantly different from zero. The

genetic correlations had high standard errors and so caution is needed when interpreting these results. The SE of heritability and correlation were estimated (Fisher *et al*., 2004) by ASReml. No bias is expected in these SE estimates from ASReml, as shown in simulation by comparison to the standard deviation of repeated estimates (Lozano-Jaramillo *et al*., 2020). These low to moderate genetic correlations indicate that LnVar is a trait that is not strongly correlated to HW and TGC. LnVar does not discriminate between positive and negative standardized deviations (Berghof *et al*., 2019b), which means that fish with constant growth can have either higher or lower than average weight. Therefore, with near zero to moderately positive genetic correlations the genetic improvement of both growth and resilience would be very well possible which could benefit performance, especially in non-aerated ponds.

Fish with low LnVar may have a better capacity to cope with disturbances and maintain their performance. A low LnVar could identify animals with less sensitivity to stressors that results in improved production, improved welfare and reduced therapeutic cost (Pottinger, 2000). Fish with lower LnVar are also expected to have a better disease resistance and better survival than fish with higher LnVar, but this needs to be confirmed by further investigation. In dairy cattle LnVar was found indicative of health traits and survival (Elgersma *et al*., 2018; Poppe *et al*., 2020). In layer chicken, a lower estimated breeding value for LnVar was predictive for lower lesion scores after avian pathogenic *Escherichia coli* inoculation (Berghof *et al*., 2019a). Lower LnVar could be indicative of the animals' ability to cope with disturbances and be less affected by stressors.

A number of studies have investigated measures of uniformity in fish with a single observation on each individual (Table 4.6). These measures are called uniformity, inherited variability, residual variance, or genetic heterogeneity of environmental variance. Measures for uniformity, based on single observations of e.g. harvest weight do not capture transient disturbances during the growth trajectory and generally have low heritability estimates ranging from 0.01 to 0.06. The GCVs of uniformity measures range from 17% to 64%, indicating that there is potential for improving uniformity by selective breeding (Janhunen *et al*., 2012; Sae-Lim *et al*., 2015; Marjanovic *et al*., 2016; Sae-Lim *et al*., 2017). However, the low heritability estimates indicate the necessity of large datasets to accurately estimate heritabilities and low accuracy to select on uniformity indicators (Hill and Mulder, 2010).

**Table 4.6** Summary of studies that investigated heritability $(h^2)$ of uniformity in fish.

| Species | Trait | method | $h^2$ | GCV | Remark | References |
|---|---|---|---|---|---|---|
| Lumpfish (*Cyclopterus limpus*) | Uniformity of body weigh | standardized body weight and log-transformed body weight | 0.01 to 0.02 | 0.46 to 0.64 | | Sae-Lim et al. (2020) |
| Nile tilapia (*Oreochromis niloticus*) | Uniformity of body weight | Square root transformed body weight | - | 0.30 to 0.44 | Information was incomplete to calculate heritability | Agha et al. (2018) |
| Atlantic salmon (Salmo *salar*) | Uniformity of growth. | Using log-transformed data and square root transformed | 0.01 to 0.04 | 0.30 to 0.52 | | Sae-Lim et al. (2017) |
| Nile tilapia (*Oreochromis niloticus*) | Uniformity of body weight | Using Standard deviation of Box-Cox transformed body weight at family by group level | 0.23 | 0.17 | The trait was defined at the group level and not directly comparable with the other studies. | Khaw et al. (2016) |
| Nile tilapia (*Oreochromis niloticus*) | Uniformity of body weight. | Using Box-cox transformed body weight | 0.03 | 0.58 | Eight observation per family per group | Marjanovic et al. (2016) |
| Rainbow trout (*Oncorhynchus mykiss*) | Body weight uniformity | Using Standardized body weight and Log-transformed data | 0.01 to 0.24 | 0.19 to 0.30 | | Sae-Lim et al. (2015) |

| Species | Trait | method | $h^2$ | GCV | Remark | References |
|---|---|---|---|---|---|---|
| Atlantic salmon (Salmo *salar*) | Genetic heterogeneity of body weight | Log-transformed, Square root transformed | 0.06 | 0.34 | | Sonesson et al. (2013) |
| Rainbow trout (*Oncorhynchus mykiss*) | Micro-environmental sensitivity of body weight (residual variation of body weight) | Using Log-transformed squared residual values | 0.02 | 0.37 | Only sire families with at least 35 offspring were selected for the analysis | Janhunen et al. (2012) |

The optimal frequency of measurements to estimate LnVar needs to be determined but this probably varies with the trait that is measured. In our study, we used five body weight records Measuring body weight measurement at five time points was found sufficient to capture the disturbances during the growth trajectory of Nile tilapia. Elgersma *et al*. (2018) and Poppe *et al*. (2020) estimated LnVar heritabilities based on 21 to 335 daily milk yield records and 50 to 350 daily milk yield records per individual, respectively. To measure LnVar, more frequent records may be required for traits that respond fast to disturbances than for traits that require some time to show a response. For a trait like milk yield daily measurements may be required because milk yield can respond quickly to disturbances and the impact may best be observed the next day. Body weight in Nile tilapia takes some time to respond to disturbances and monthly, bi-weekly or weekly measurements could be sufficient to capture disturbances.

While 5 measurements were sufficient for LnVar based on growth in Nile tilapia, this still required repeated phenotyping of individuals which is consuming and can be stressful on the fish. Methods to perform automated phenotyping and image analysis are rapidly developing (Yang *et al*., 2021) which will make multiple measurements per individual over time easier. Automated phenotyping is non-invasive to fish and in time it may provide an automatic and effective size measurement (Li *et al*., 2020). Technological developments in automated phenotyping are expected to facilitate the application of resilience traits based on multiple measurements over time.



In aquaculture, constancy of growth leads to more uniformity in fish sizes which is important for biomass estimation, feeding decisions and to schedule harvesting. The heritabilities of 0.10 to 0.12 for LnVar show that constancy of growth could be improved by selective breeding. Improving uniformity in Nile tilapia by selection would bring economic benefits to the farmer, leads to less stress due to the reduced need of size grading and reduced competition among fish (Omasaki *et al*., 2017). More accurate biomass estimation from less variable growth rates also results in more optimal feeding regimes and less feed waste. At cohort/cage level, resilient fish may have more constant feed intake over time, waste less feed, and would therefore be more efficient. FE can vary widely in Nile tilapia production systems (Mengistu *et al*., 2020b), and the economic value of improving FCR by selection is considerable (0.41 US$/kg production/σa; Omasaki *et al*., 2017). Selecting for more resilient fish could therefore lead to a correlated response in FE

and in that case improving resilience would also have a positive effect on the environmental impact of fish farming (Besson *et al*., 2016).

In conclusion, substantial additive genetic variance was found for LnVar in the aerated and non-aerated ponds and this can be exploited by selective breeding in Nile tilapia. Favorable genetic correlations of LnVar with health, survival and feed efficiency may be expected but this needs to be confirmed in further research. To improve resilience together with growth we recommend that fish breeding programs collect repeated records on body weight and use of sib testing in non-aerated pond with genomic selection.

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CHAPTER 5

# 5

# Heritable variation in swimming performance in Nile tilapia (*Oreochromis niloticus*) and negative genetic correlations with growth and harvest weight

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## Abstract

Nile tilapia is predominantly produced in smallholder ponds without aeration. We hypothesize that Nile tilapia with high oxygen uptake efficiency ($O_2$UE) may perform better under these conditions than Nile tilapia with low $O_2$UE. Critical swimming speed ($U_{crit}$ in cm $s^{-1}$) is a potential indicator for $O_2$UE. Our objectives were to estimate variance components for $U_{crit}$ and fish size at swim testing early in life, and genetic correlations ($r_g$) between $U_{crit}$ with harvest weight (HW) and daily growth coefficient (DGC) later after grow-out in a non-aerated pond. Substantial heritability was found for absolute $U_{crit}$ (0.48). The estimated $r_g$ between absolute $U_{crit}$ and fish size at testing were all strong and positive (range 0.72 - 0.83). The estimated $r_g$ between absolute $U_{crit}$ and HW, and absolute $U_{crit}$ and DGC were -0.21 and -0.63 respectively, indicating that fish with higher absolute $U_{crit}$ had lower growth in the non-aerated pond as compared to fish with lower absolute $U_{crit}$. These results suggest a juvenile trade-off between swimming and growth performance where fish with high $U_{crit}$ early in life show slower growth later under conditions of limited oxygen availability. We conclude that $U_{crit}$ in Nile tilapia is heritable and can be used to predict growth performance.

## 5.1 Introduction

Nile tilapia (*Oreochromis niloticus*) is predominantly produced in smallholder tilapia ponds without aeration. In non-aerated ponds dissolved oxygen (DO) drops below critical level (3 mg $l^{-1}$) (Stickney, 2017) during night. Low DO in smallholder farms negatively affects Nile tilapia growth (Mengistu *et al*., 2020a). It may be expected, therefore, that Nile tilapia with high oxygen uptake efficiency may grow better under these conditions than Nile tilapia with low oxygen uptake efficiency. As critical swimming speed ($U_{crit}$) may reflect the oxygen uptake efficiency, the hypothesis is that fish with high $U_{crit}$ will grow better under conditions where oxygen is limiting.

A high throughput method to assess the individual variation in oxygen uptake efficiency is by subjecting fish to exhaustive exercise in a critical swimming challenge test. In this test, swimming speeds are incrementally increased at prescribed intervals until fish stop swimming and fatigue (Brett, 1964; Plaut, 2000). Individual fish fatigue when swimming at a specific speed interval for a certain period, from which the $U_{crit}$ (Brett, 1964) can be determined. Recently we have developed and applied such tests for gilthead seabream (*Sparus aurata*) and Atlantic salmon (*Salmo salar*) (Palstra *et al*., 2020a). Oxygen uptake is maximal at $U_{crit}$, although the anaerobic component by fast skeletal muscle increases when nearing $U_{crit}$ (Videler, 1993). Near $U_{crit}$, the metabolic demand for oxygen is greater than can be provided by ventilatory and circulatory systems (Jones and Randall, 1978). Fish that are able to consume more oxygen can swim faster, or reverse for the connection that we are interested in: faster swimming fish have higher oxygen uptake efficiency. Particularly for tilapia, the link between $U_{crit}$ and maximal oxygen consumption may be strong because tilapia has a high $U_{crit}$ (4.94 ± 0.45 BL $s^{-1}$ for ~15 cm fish) and a very high maximum metabolic rate (McKenzie *et al*., 2003). Hence, $U_{crit}$ could be a good indicator of oxygen uptake efficiency of individual tilapia.

The heredity of athletic performance has received considerable research attention in dog (Kim *et al*., 2018), horse (Thorén Hellsten *et al*., 2006; Hill *et al*., 2013) and human (Issurin, 2017). Genetic parameter estimates for swimming performance in fish are scarce, but suggest that swimming performance has a heritable component. Broad sense heritabilities (i.e. not corrected for dominance and epistatic interaction effects) (Falconer and Mackay, 1996) of swimming performance were estimated by Garenc *et al*. (1998) in stickleback (*Gasterosteus aculeatus*), by Hurley and Schom (1984) in Atlantic Salmon and by Nicoletto (1995)

in guppy (*Poecilia reticulata*). More recently, Vandeputte *et al*. (2016) estimated the additive genetic variance component for relative $U_{crit}$ ($U_{crit}$ divided by standard length) in European sea bass (*Dicentrarchus labrax*) and found a heritability of 0.55, with a negative genetic correlation with body weight.

We therefore aimed first to estimate variance components for swimming performance in Nile tilapia expressed as $U_{crit}$ and to estimate the genetic correlation between $U_{crit}$ and fish size at swim testing early in life. Next, tested fish were stocked in a non-aerated pond and grown to harvest weight, to determine the genetic correlations between $U_{crit}$ early in life and harvest weight (HW) and daily growth coefficient (DGC) later in life.

## 5.2 Materials and methods

### 5.2.1 Ethics statement

This study utilised phenotypic data collected as part of the GIFT selective breeding program managed by WorldFish at the Aquaculture Extension Centre of the Malaysian Department of Fisheries at Jitra, Kedah State, Malaysia (6°15’32°N; 100°25’47°E). This study was approved by the internal WorldFish ethics committee. All fish in the GIFT breeding population are managed in accordance with the Guiding Principles of the Animal Care, Welfare and Ethics Policy of WorldFish.

#### 5.2.2 Experimental fish

Nile tilapia of the Genetically Improved Farmed Tilapia (GIFT) strain from generation 18 was used in this experiment. The 60 full sib and half sib families were produced using 31 males and 58 females, of which two females were used twice with different males. The planned mating ratios were one male to at least two females. However, the successful mating were: 12 males each mated with one female (resulting in 12 full sib families), 12 males each mated with two females (resulting in 12 half sib groups equivalent to 24 full sib families), 4 males each mated with 3 females (four half sib groups equivalent to 12 full sib families) and 3 males each mated with 4 females (three half sib groups equivalent to 12 full sib families). Each full sib family was reared separately in a hapa (fine mesh net enclosure) set up in an earthen pond.

The image analysis was done as described previously by (Mengistu *et al*., 2020b). In total 1,500 photographs were loaded into tpsUtil software (Rohlf, 2017b) and digitized for six landmarks using tpsDIG 2.30 (Rohlf, 2017a). Landmarks 1 and 2 were on the 0 and 20 cm marks on the ruler which was photographed together

with the fish for scaling. The landmarks 3 and 4 were used to measure standard length, the distance between the tip of the snout to the base of caudal fin. The landmarks 5 and 6 were the dorsal and ventral landmarks where the distance is maximum. These landmarks were used to calculate height, the maximum dorso-ventral distance (Figure 5.1). To obtain the distance between the Cartesian coordinates, these landmarks were analysed in R software using geomorph-package version 3.0.7 (Adams *et al*., 2018) and the true distance in cm was computed based on the reference scale.


Figure 5.1 Nile tilapia with landmarks 1:6. Landmarks 1 and 2 marks a reference scale of 20 cm length, landmarks 3 and 4 represent the snout and base of the caudal fin, respectively, landmarks 5 and 6 were used to measure height (maximum dorso-ventral length) of the experimental fish.

### 5.2.3 Swim test experiment

The swim test was done in 30 working days, one swim test per day. Thirty to 35 relatively bigger fingerlings from 60 full sib families were selected, PIT tagged and housed in a tank. Three weeks after PIT tagging, 25 fish in a range from 5 to 10 cm standard length at swim testing (SLtest, in cm) from each of the 60 full sib families were measured using a ruler with a centimetre scale, weighed (Wtest, in g) and photographs were made, one day before the swim test. The SLtest and height at swim testing (Htest, in cm) of the fish used in our analysis were obtained from the photographs of each fish using image analysis. The number of fish tested per family was 25 and the number of fish per test was 50 fish. Therefore, we tested either 10

fish from 5 families or 5 fish from 10 families which resulted in all 25 fish from each family being tested in three consecutive days.

To determine the $U_{crit}$, a Brett type (rectangular oval shape raceway) swim flume of 230 cm length and 90 cm width with a water depth of 40 cm was used (Palstra, 2016). Water current was created using a Minn Kota® Terrova 80 lbs propeller. The propeller has 10 speed settings, in this experiment speed levels from 2 to 10 were used. Table 5.7 (See Appendix A) provides the flow speeds measured at each of the settings. As the assessment of $U_{crit}$ requires all fish to fatigue, this experimental set-up could be applied for early life testing at small size and not for older and larger fish.

Feeding was stopped 24 hrs before the beginning of the swim testing. The fish were acclimatised for one hour in the swimming flume without flow. After acclimation, the propeller was turned on to induce swimming at the second setting. The time at each setting was fixed at 30 minutes and flow increments continued until all fish fatigued. At each setting, the average water flow velocity was recorded using a FP111 global water flow probe (FP111, Global Water, USA). The swim test could take maximally 4.5 hrs, with 9 propeller speed levels. A fish fatigued when it touched the back fence and could not be stimulated to continue swimming. Each fatigued fish was scooped out immediately and PIT tag number and time at fatigue were recorded.

The mean dissolved oxygen in the tank just before resuming the swim test was 5.6 ± 0.4 mg $l^{-1}$ (71.2% saturation), ranging from 4.9 to 6.4 mg $l^{-1}$, and during the swim test it was 7.6 ± 0.4 mg $l^{-1}$ (97.6% saturation), ranging from 6.4 to 8.8 mg $l^{-1}$. The mean water temperature in the tank just before resuming the swim test was 27.7 ± 0.6°C, ranging from 26.5 to 28.5°C, and during the swim test it was 28.3 ± 0.6°C, ranging from 26.5 to 29.9°C.

### 5.2.5 Calculation of critical swimming performance and surface area

Absolute and relative critical swimming speed ($U_{crit}$) was used as a measure of swimming performance and calculated according to Brett (1964):

$$\text{Absolute } U\text{crit} = \mathrm{U}_{-1} + (\frac{\mathrm{t}}{\Delta \mathrm{t}})\Delta \mathrm{U} \quad (1)$$

$$\text{Relative } U\text{crit} = (\mathrm{U}_{-1} + \left(\frac{\mathrm{t}}{\Delta \mathrm{t}}\right)\Delta \mathrm{U})/\mathrm{SLtest} \quad (2)$$

where $U_{-1}$ is the highest velocity maintained for the prescribed period in $cm\ s^{-1}$, $\Delta U$ is velocity increment in $cm\ s^{-1}$, $t$ is time to fatigue at final velocity level in minutes, $\Delta t$ is the time each velocity level is maintained at (=30 minutes) and $SLtest$ is standard length of fish at swim testing in cm. Figures were produced using Minitab (Minitab 17 Statistical Software, 2010).

Surface area at swim testing (SAtest) of Nile tilapia is similar to the area of an ellipse and was calculated as:

$$SA = \frac{1}{4}\pi * SLtest * Htest \quad (3)$$

### 5.2.5 Grow-out in the non-aerated pond

Swim tested fish were stocked in a non-aerated pond for grow-out. The pond size was 500 $m^2$ and the socking density was 3 fish per $m^2$. During the grow-out period, DO was above 5 mg $l^{-1}$ except from 9:00 pm to 9:00 am when DO would drop below 3 mg $l^{-1}$. Fish were weighed and photographed before stocking into the non-aerated pond. The mean weight at cultivation start (Wstart) was 10.8 g and the coefficient of variation (CV) was 23.7%. The fish were fed commercial feed at a rate of 3 to 5% of their body weight depending on their sizes, with the percentage of feed decreasing with size. The fish were harvested after 145 or 146 days of grow-out. Each fish was weighed at harvest. At harvest the sex of a random half of the fish (763 fish) were determined.

Daily growth coefficient (Iwama and Tautz, 1981; Trọng *et al*., 2013) was computed as:

$$DGC = \left[\frac{\sqrt[3]{HW} - \sqrt[3]{SW}}{time\ in\ days}\right] \times 100 \quad (4)$$

where HW is harvest weight and Wstart is stocking weight.

### 5.2.6 Statistical analysis

Phenotypic and genetic parameters were estimated using ASReml version 4.1(Gilmour *et al*., 2015). The following animal model was used:

$$\boldsymbol{y} = \mathbf{Xb} + \mathbf{Z_1 a} + \mathbf{Z_2 c} + \mathbf{e} \quad (5)$$

where $\boldsymbol{y}$ is a vector of either absolute $U_{crit}$, or relative $U_{crit}$ in the univariate model, $\mathbf{b}$ is the vector of fixed effects, that is test day and sex fitted as class variable for relative $U_{crit}$ while for absolute $U_{crit}$ three different models were fitted with: 1) test

day and sex fitted as class variables, 2) test day and sex as class variables and Wtest as a covariate and 3) test day and sex as class variables and SLtest as a covariate, sex was not significant in all the three models; therefore, sex was removed from the models; $\mathbf{a}$ is a vector of additive genetic effects, $\mathbf{c}$ is a vector of environmental effects common to full sibs ('hapa effect'), and $\mathbf{e}$ is a vector of residual effects. The $\mathbf{X}$, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are design matrices assigning phenotypic values to the levels of fixed effect, additive genetic and common environmental effects, respectively. The effect of sex was also not significant when subset of the data with only 763 sexed fish was analysed.

Bivariate models were used to estimate the phenotypic and genetic correlations between absolute $U_{\text{crit}}$, and traits such as Wtest, SLtest, Htest, SAtest, HW and DGC. In the bivariate models test day and sex were fitted as a class variable for absolute $U_{\text{crit}}$, age at harvest was fitted as a covariate for HW and sex was fitted as a class variable for DGC. Common environmental effect was fitted as a random variable to all the traits except for DGC in the bivariate model absolute $U_{\text{crit}}$ and DGC. The bivariate model with absolute $U_{\text{crit}}$ and DGC did not converge when a common environmental effect was fitted as a random effect on both traits. The additive genetic effects were normally distributed as $\mathrm{N} = \left( \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \mathbf{A} \otimes \begin{bmatrix} \sigma^2_{a,1} & r_{a,12}\sigma_{a,1}\sigma_{a,2} \\ r_{a,21}\sigma_{a,2}\sigma_{a,1} & \sigma^2_{a,2} \end{bmatrix} \right)$, where $\mathbf{A}$ is the numerator genetic relationships matrix and $\sigma^2_{\mathrm{a},1}$ ($\sigma^2_{\mathrm{a},2}$ ) being the additive genetic variance of trait 1(2) and $r_{a,12(21)}$ being the genetic correlation between trait 1 and 2. The pedigree depth was 18 generations, i.e. from the current generation G18 all the way back to the first generation of GIFT in WorldFish, Malaysia. The common environmental effects were normally distributed as $\mathrm{N} = \left( \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \mathbf{I} \otimes \begin{bmatrix} \sigma^2_{c,1} & r_{c12}\sigma_{c,1}\sigma_{c,2} \\ r_{c21}\sigma_{c,2}\sigma_{c,1} & \sigma^2_{c,2} \end{bmatrix} \right)$, where $\mathbf{I}$ being an identity matrix and $\sigma^2_{\mathrm{c},1}$ ($\sigma^2_{\mathrm{c},2}$ ) being the common environmental variance of trait 1(2) and $r_{c,12(21)}$ being the common environmental correlation between trait 1 and 2. The residual effects were normally distributed as $\mathrm{N} = \left( \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \mathbf{I} \otimes \begin{bmatrix} \sigma^2_{\mathrm{e},1} & r_{e,\,12}\sigma^2_{\mathrm{e},1}\sigma^2_{\mathrm{e},2} \\ r_{e,21}\sigma^2_{\mathrm{e},2}\sigma^2_{\mathrm{e},\,1} & \sigma^2_{\mathrm{e},2} \end{bmatrix} \right)$, where $\sigma^2_{\mathrm{e},1}$ ($\sigma^2_{e,2}$ ) being the residual variance of trait 1(2) and $r_{e,12(21)}$ being the residual correlation between trait 1 and 2.

Heritability $(\mathrm{h}^2)$ and the ratio of common environmental variance $(\mathrm{c}^2)$ to phenotypic variance $(\sigma^2_p)$ of each trait was computed as $\mathrm{h}^2 = \sigma^2_{\mathrm{a}}/\sigma^2_{\mathrm{p}}$ and $\mathrm{c}^2 = \sigma^2_{\mathrm{c}}/\sigma^2_{\mathrm{p}}$, respectively. The significance of the random effects were tested using

loglikelihood ratio test with one degree of freedom (Lynch and Walsh, 1998).To test whether the genetic correlation is larger than zero, model without constraining the covariance was tested against a model where the covariance was constrained to zero. The full model, i.e. a model with both common environmental effects and additive genetic effects as random effects, were tested against a reduced model, i.e. a model with either only common environmental effect or additive genetic effects as a random effect. The common environmental variances were not significantly different from zero ($P > 0.05$) except for relative $U_{crit}$ ($P = 0.006$). The most likely reason that the common environmental effect was not significant in most cases was because of the almost complete confounding of sire genetic, dam genetic and common environmental effects in the experiment. This reflected the fact that 24 of the males were mated to one or two females resulting in 12 families with no half sib families and 12 families with only one half sib family (40% of the total families), making genetic and common environmental effects difficult to disentangle. Although, common environmental effects were not significant for most traits, common environmental effects explained a substantial part of the phenotypic variance and were kept in the model, to prevent overestimation of the additive genetic variance. The loglikelihood for the bivariate model with absolute $U_{crit}$ and DGC did not converge when common environmental effect was fitted as a random effect on both traits. Therefore, the common environmental effect was fitted as a random effect only on absolute $U_{crit}$ in the bivariate model with absolute $U_{crit}$ and DGC.



## 5.3 Results

### 5.3.1 Biometric data

In total 1,500 fish were swim tested and stocked in the non-aerated pond. Out of the swim tested 1,500 fish, the swimming performance data of seven fish were missing and resulted in 1,493 $U_{crit}$ records. The descriptive statistics for age at swim testing (Agetest), Wtest, SLtest, Htest, SAtest, weight at cultivation start, and HW and DGC later in life are presented in Table 1. Out of the stocked 1,500 fish, ultimately 1,199 were harvested which is equivalent to 79.9% survival.

### 5.3.2 Swimming performance

The mean absolute $U_{crit}$ and relative $U_{crit}$ were 69.1 ± 5.5 cm $s^{-1}$ and 9.7 ± 0.9 SL $s^{-1}$, respectively (Table 5.1). Absolute $U_{crit}$ and relative $U_{crit}$ values showed normal distributions (Figure 5.2). There was substantial variation in swimming performance between family means (Figure 5.3), indicating existence of genetic variation.

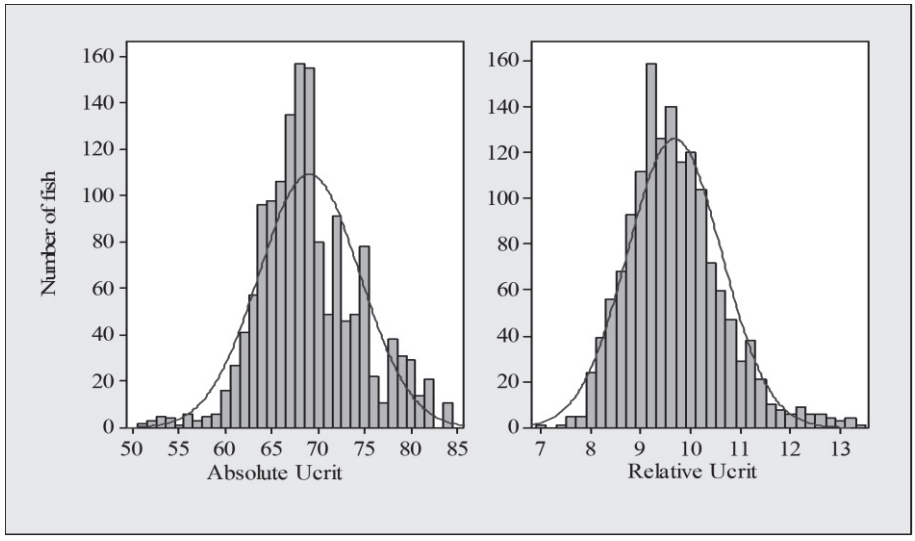


Figure 5.2 Distribution of absolute $U_{crit}$ (cm $s^{-1}$) and relative $U_{crit}$ (SL $s^{-1}$).

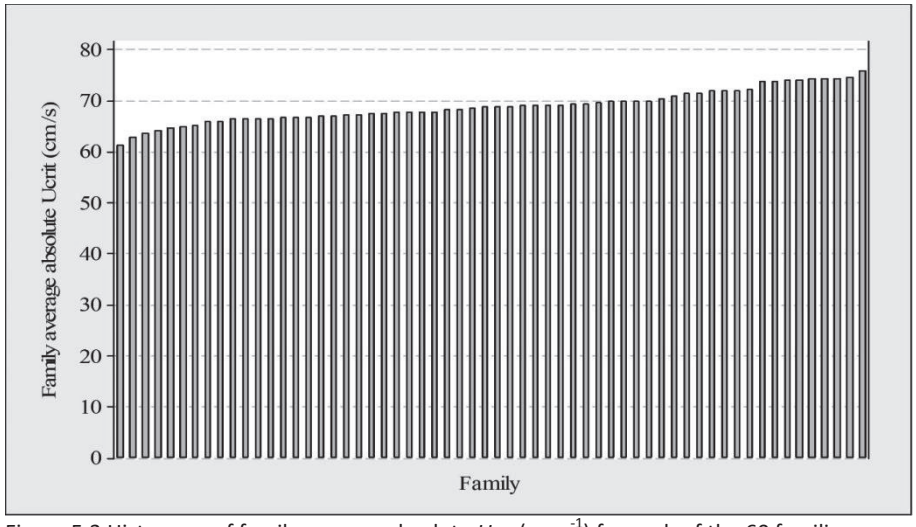


Figure 5.3 Histogram of family average absolute $U_{crit}$ (cm $s^{-1}$) for each of the 60 families.

### 5.3.3 Genetic parameters

Variances, heritability and the ratio of common environmental variance to the phenotypic variance ($c^2$) effect for absolute and relative $U_{crit}$ are presented in Table 5.2. The heritability for absolute $U_{crit}$ was 0.48 ± 0.17 when Wtest or SLtest was not fitted in the model as a covariate. The heritability for absolute $U_{crit}$ was 0.41 ± 0.16 when SLtest was fitted as a covariate and 0.44 ± 0.16 when Wtest was fitted as a

covariate. The heritability for relative $U_{crit}$ (0.15 ± 0.13) was low. The common environmental effect explained a small proportion of the phenotypic variance (0.02 to 0.04) for absolute $U_{crit}$, while the contribution was substantial for the phenotypic variance of relative $U_{crit}$ (0.13). The analyses with absolute $U_{crit}$ with Wtest or SLtest in the model as a covariate showed that $U_{crit}$ contained considerable heritable variation even when corrected for body size.

The additive genetic variance contributed a significant proportion to the phenotypic variance of absolute $U_{crit}$ ($P$ = 0.000) and absolute $U_{crit}$ when either Wtest ($P$ < 0.001) or SLtest ($P$ = 0.001) was fitted in the model as covariate, while the contribution was not significant for relative $U_{crit}$ ($P$ = 0.175). The contribution of common environmental effect to the phenotypic variance of absolute $U_{crit}$ ($P$ = 0.584) and for absolute $U_{crit}$ when either Wtest ($P$ = 0.384) or SLtest were fitted as covariates ($P$ = 1.000) were not significant, while the contribution to the phenotypic variance of relative $U_{crit}$ ($P$ = 0.007) was significant.

**Table 5.1** Number of fish (N), mean, standard deviation (SD), coefficient of variation (CV and minimum and maximum values for critical swimming speed ($U_{crit}$)absolute and relative, age at swim tesing (Agetest), body weight at swim testing (Wtest), standard length at swim testing (SLtest), and body height at swim testing (Htest), surface area at swim testing (SAtest), stocking weight (SW), harvest weight (HW) and daily growth coefficient (DGC).

| | N | mean | SD | CV | min | max |
|---|---|---|---|---|---|---|
| Absolute $U_{crit}$ cm $s^{-1}$ | 1,493 | 69.1 | 5.5 | 7.9 | 50.6 | 83.8 |
| Relative $U_{crit}$ SL $s^{-1}$ | 1,493 | 9.7 | 0.9 | 9.8 | 6.9 | 13.3 |
| Agetest (days) | 1500 | 86.8 | 12.1 | 13.9 | 65 | 139 |
| Wtest (g) | 1,500 | 10.8 | 2.6 | 23.7 | 4.8 | 20.1 |
| SLtest (cm) | 1,500 | 7.2 | 0.6 | 8.0 | 5.3 | 8.9 |
| Htest (cm) | 1,500 | 2.7 | 0.2 | 9.0 | 2.1 | 3.5 |
| SAtest ($cm^2$) | 1,500 | 15.3 | 2.5 | 16.4 | 9.2 | 24.0 |
| SW (g) | 1,199 | 27.4 | 15.1 | 47.8 | 7.3 | 94.2 |
| HW (g) | 1,199 | 417.7 | 88.1 | 21.1 | 153.4 | 778.9 |
| DGC | 1,199 | 3.1 | 0.3 | 10.8 | 1.7 | 3.8 |

Table 5.2 Additive genetic variance ($\sigma_a^2$), common environmental variance ($\sigma_c^2$), phenotypic variance ($\sigma_p^2$), heritability and common environmental effect ($c^2$) of absolute and relative critical swimming speed ($U_{crit}$).

| | $\sigma^2_a$ | $\sigma^2_c$ | $\sigma^2_p$ | Heritability | $c^2$ |
|---|---|---|---|---|---|
| Absolute $U_{crit}$* | 8.90 | 0.43 | 18.45 | 0.48±0.17 | 0.02±0.05 |
| Absolute $U_{crit}$** | 6.71 | 0.59 | 16.20 | 0.41±0.16 | 0.04±0.05 |
| Absolute $U_{crit}$*** | 6.79 | 0.55 | 16.09 | 0.44±0.16 | 0.03±0.05 |
| Relative $U_{crit}$ | 0.08 | 0.07 | 0.55 | 0.15±0.13 | 0.13±0.06 |

*Absolute $U_{crit}$ without body weight or standard length at swim testing in the model.

**Absolute $U_{crit}$ when standard length at swim testing was included in the model as covariate.

***Absolute $U_{crit}$ when body weight at swim testing was included in the model as covariate.

The estimated genetic correlations ($r_g$) and phenotypic correlations ($r_p$) between absolute $U_{crit}$ and Wtest, SLtest, Htest, SAtest, HW and DGC are presented in Table 5.3. The genetic correlations were significant ($P$ < 0.05) except for the genetic correlation between $U_{crit}$ early in life and HW later in life ($P$ = 0.507) based on likelihood ratio test (Lynch and Walsh, 1997).The estimated $r_g$ and $r_p$ correlations between absolute $U_{crit}$ and Wtest were 0.78 and 0.44, respectively. The less than one $r_g$ between absolute $U_{crit}$ and Wtest indicates the presence of genetic variance in absolute $U_{crit}$ that is not explained by Wtest. Genetic and phenotypic correlations with the other size measurements SLtest, Htest and SAtest were very similar. Fish with higher absolute $U_{crit}$ had lower HW and DGC after grow-out in a non-aerated pond. The estimated $r_g$ and $r_p$ between absolute $U_{crit}$ and HW were -0.21 and -0.04, respectively and the estimated $r_g$ and $r_p$ between absolute $U_{crit}$ and DGC were -0.63 and -0.24, respectively. The negative genetic correlations between $U_{crit}$ and HW and between absolute $U_{crit}$ and DGC indicate that fish with higher absolute $U_{crit}$ perform less in terms of HW and DGC compared to fish with lower absolute $U_{crit}$.

Table 3: Genetic and phenotypic correlations between absolute critical swimming speed ($U_{crit}$) and body weight at swim testing (Wtest), standard length at swim testing (SLtest) height at swim testing (Htest), surface area at swim testing (SAtest), harvest weight (HW) and daily growth coefficient (DGC).

| Trait | $r_g$ | $r_p$ |
|---|---|---|
| Wtest | 0.78±0.18 | 0.44±0.05 |
| SLtest | 0.83±0.19 | 0.43±0.05 |
| Htest | 0.72±0.22 | 0.37±0.05 |
| SAtest | 0.83±0.18 | 0.42±0.05 |
| HW | -0.21±0.29 | -0.04±0.06 |
| DGC | -0.63±0.15 | -0.24±0.07 |

$U_{crit}$ was estimated in a bivariate model without Wtest or SLtest as covariate.

## 5.5 Discussion

Our objectives were to estimate variance components for swimming performance in Nile tilapia, assessed as critical swimming speed ($U_{crit}$) early in life, and to estimate the genetic correlation between $U_{crit}$ and body size early in life, and harvest weight (HW) and Daily Growth Coefficient (DGC) later in life after a grow-out period in a non-aerated pond. For the first time, we show with a large-scale experiment that swimming performance is heritable in Nile tilapia, and that the genetic correlation with harvest weight is strongly negative, even when corrected for body size at testing. The heritabilities, the genetic correlations, methodology and the practical application of a swimming performance test in breeding programs are discussed.



This study shows the existence of heritable variation in critical swimming performance with a moderate heritability of 0.41 - 0.48. Our heritability estimate for $U_{crit}$ early in life is in the same range as reported previously for other species and for similar traits (for summary see Table 5.4). Of the four studies that estimated genetic parameters for swimming performance in fish, only the study that assessed the burst swimming performance trait is not comparable with $U_{crit}$ in our study (Garenc *et al*., 1998). Our heritability estimate for relative $U_{crit}$ (0.15) was not significantly different from zero, which is different from the heritability of 0.55 for relative maximum swimming speed in European sea bass *Dicentrarchus labrax* (Vandeputte *et al*., 2016). The difference in heritability of relative $U_{crit}$ might be due to a species specific difference, particularly reflecting the high or long body shape of tilapia and sea bass, respectively.

**Table 4.6** Summary of the studies that estimated heritability for different swimming performance traits, genetic phenotypic correlation between swimming performance and body weight, and between swimming performance and body length.

| Trait | Species | Comments | $h^2$ | Genetic ($r_g$) and phenotypic ($r_p$) correlations | Reference |
|---|---|---|---|---|---|
| Critical Swimming speed | Guppy (*Poecilia reticulata*) | Measured by increasing the water velocity every 3 minutes until the fish fatigued<br>16 full sib families were used (96 fish in total) | 0.24 ± 0.19 | Not given | (Nicoletto, 1995) |
| Swimming stamina (similar trait with critical swimming speed) | Atlantic salmon (*Salmo salar*) | Measured as the total time the fish swam until fatigue by increasing water velocity incrementally every 4 minutes<br>11 full sib families were used (129 fish in total) | 0.24 ± 0.16 | $r_g$ = 0.23 and $r_p$ = 0.85 (between stamina and body weight)<br>$r_g$ = -0.14 and $r_p$ = 0.18 (between stamina and body length) | (Hurley and Schom, 1984) |
| Absolute burst swimming (cm/s) (not comparable with critical swimming speed) | Threespine stickleback (*Gasterosteus aculeatus*) | Measured as distance swam in 160ms using video recording,<br>2 months old 193 fish from 25 full sib families were used | 0.41* | Not given | (Garenc *et al.*, 1998) |

| Trait | Species | Comments | $h^2$ | Genetic ($r_g$) and phenotypic ($r_p$) correlations | Reference |
|---|---|---|---|---|---|
| Relative burst swimming (body length/s) (not comparable with critical swimming speed) | Threespine stickleback | Measured as distance swam in 160ms using video recording 2 months old 193 fish from 25 full sib families were used | 0.37 | Not given | (Garenc *et al.*, 1998) |
| Absolute burst swimming (cm/s) (not comparable with critical swimming speed) | Threespine stickleback | Measured as distance swam in 160ms using video recording 3.6 months old 181 fish from 25 full sib families were used | 0.02 | Not given | (Garenc *et al.*, 1998) |
| Relative burst swimming (body length/s) (not comparable with critical swimming speed) | Threespine stickleback | Measured as distance swam in 160ms using video recording 3.6 months old 181 fish from 25 full sib families were used | 0.00 | Not given | (Garenc *et al.*, 1998) |
| Relative maximum sustained speed (similar trait with relative critical swimming speed) | European sea bass (*Dicentrarchus labrax*) | Measured as the last fully accomplished water velocity 547 fish from 366 full sib families, paternal and maternal half sib families were used | 0.55 | $r_g$ = -0.64 and $r_p$ = -0.56 between relative maximum sustained speed and body weight | (Vandeputte *et al.*, 2016) |

| Trait | Species | Comments | $h^2$ | Genetic ($r_g$) and phenotypic ($r_p$) correlations | Reference |
|---|---|---|---|---|---|
| Absolute critical swimming speed | Nile tilapia (*Oreochromis niloticus*) | Explained in section 2.3 of this paper<br>1493 fish, full sib and half sib families<br>Absolute $U_{crit}$ without including body weight/standard length in the model as covariate | 0.48 | $r_g$ = 0.87 and $r_p$ = 0.44 between absolute $U_{crit}$ and body weight | This study |
| Absolute critical swimming speed | Nile tilapia | 1493 fish, full sib and half sib families<br>Absolute $U_{crit}$ when body weight was included in the model as covariate | 0.42 | | This study |
| Absolute critical swimming speed | Nile tilapia | 1493 fish, full sib and half sib families<br>Absolute $U_{crit}$ when standard length was included in the model as covariate | 0.41 | | This study |
| Relative critical swimming speed | Nile Tilapia | 1493 fish, full sib and half sib families | 0.15 | | This study |

Species specific differences also exist in relation between $U_{crit}$ and body size. Absolute $U_{crit}$ was genetically strongly correlated with body weight at swim testing (0.78). This is higher than the estimated genetic correlation between swimming stamina and body weight in Atlantic salmon (0.23) (Hurley and Schom 1984). The genetic correlation between absolute $U_{crit}$ and standard length (0.83) was also different from the estimated $r_g$ between swimming stamina and fork length in Atlantic salmon (-0.14) (Hurley and Schom 1984).

To the best of our knowledge there are no studies on $r_g$ between absolute $U_{crit}$ and traits such as Htest, SAtest, HW and DGC with which to compare our results. In our study, the $r_g$ estimates between absolute $U_{crit}$ and Htest, between absolute $U_{crit}$ and SAtest were 0.72 and 0.83, respectively. These strong genetic correlations between absolute $U_{crit}$ and SLtest and Wtest early in life show that larger fish swim faster in absolute terms.

The estimated $r_g$ values between absolute $U_{crit}$ and HW and absolute $U_{crit}$ and DGC were -0.21 and -0.63, respectively, meaning that fish with high $U_{crit}$ at testing had lower DGC and HW later in life. These negative genetic correlations do not support our hypothesis that Nile tilapia with higher $U_{crit}$, reflecting higher oxygen uptake efficiency, are those that perform better in terms of weight increase in non-aerated ponds where hypoxia is frequent. Instead, the negative $r_g$ shows that fish with higher $U_{crit}$ early in life show less body weight increase later in life. These data do not provide insight on fish body shape and composition at slaughter size. For example, it may be that fish with higher $U_{crit}$ are the leaner fish later as compared to fish with lower $U_{crit}$. Fish with lower $U_{crit}$ may be heavier but not necessarily have more fillet mass. Results of a $U_{crit}$ test in Gilthead sea bream (*Sparus aurata*), also a high bodied fish, showed that the (residual) $U_{crit}$ was negatively correlated with fillet mass suggesting that fast swimmers build lower fillet mass later in life (Palstra *et al*., 2020b). A plausible explanation for our results may be the existence of a juvenile trade-off between swimming and growth performance where fish with high $U_{crit}$ early in life show slower growth later. Young juveniles may choose to either swim fast or grow fast, representing, for instance, two anti-predator strategies: to be able to escape predators or to become too large to be eaten rapidly. Studies have shown that a trade-off between growth rate and locomotor performance can exist (Billerbeck *et al*. 2001), for instance during accelerated growth (Lee *et al*., 2010) which can negatively influence muscle cellularity and development (Galloway *et al*., 1999; Johnston, 2003). Indeed, fast-growing growth hormone (GH) transgenic carp (Li *et al*., 2007) had lower critical swimming

performance than non-transgenic controls. Fast-growing GH transgenic salmon had similar critical swimming speeds than non-transgenic controls but was also able to consume considerable more oxygen (Stevens *et al*., 1998) and may thus have compensated for lower critical swimming performance.

In our study, 1,493 fish were used to estimate genetic parameters. The mating ratio used to produce the experimental fish was 1 male to 1 – 4 females, which gave full sib and half sib families. The previous studies that estimated genetic parameters used a much lower number of fish (range 96 -129) as compared to our study and estimated broad sense heritability using full sib families (Table 5.4) (Hurley and Schom, 1984; Nicoletto, 1995). The much larger sample size gave a much higher precision of estimates of narrow-sense heritability. Furthermore, broad-sense heritability estimates are biased estimates of narrow-sense heritabilities, because broad-sense heritabilities contain non-additive genetic variation due to dominance and epistasis that is not heritable from parent to offspring and may contain common environmental effects, because in such full sib designs estimation of common environmental effects is not feasible (Lozano-Jaramillo *et al*., 2020). Narrow-sense heritability, however, is the ratio of additive genetic variance to phenotypic variance (Falconer and Mackay, 1996) and therefore a better indication of the proportion of genetic variation that is transmitted to the next generation. In our study, we used half sib families that enabled us to estimate narrow sense heritability. Similarly, Vandeputte *et al*. (2016) estimated a narrow sense heritability using half sib families based on 547 fish. The main difference in the swimming performance trait between our study and Vandeputte *et al*. (2016) was that these authors did not include the last water velocity level that the fish did not fully complete. Besides the species difference mentioned earlier, also the number of fish and the way the swimming performance was calculated could provide additional explanation for the difference in the parameter estimates between our study and Vandeputte *et al*. (2016).

Critical swimming speed can be calculated in four different ways: as absolute $U_{crit}$, with or without Wtest or SLtest as covariate in the model, as relative $U_{crit}$, or as residual $U_{crit}$ which is the difference in $U_{crit}$ of an individual fish with the predicted value on basis of its length (Palstra *et al*., 2020b). Analysing absolute $U_{crit}$ without a covariate for either Wtest or SLtest, has the highest additive genetic variance, but part of that genetic variance is due to genes affecting body size. The use of fish with similar body weight at similar SLtest is practically difficult as the variation is considerable; in our experiment the Wtest was from 4.8 to 20.1 g for fish from 5.4

to 10 cm SL. Therefore, it is important to account for Wtest or SLtest in the analysis to be able to estimate heritable variation in $U_{crit}$ independent of body size.

Relative $U_{crit}$ is a ratio of $U_{crit}$ to SLtest for which the estimated heritability was not significantly different from zero in our study. Relative $U_{crit}$ is a ratio trait and therefore the genetic variance becomes a complex function of absolute $U_{crit}$ and SLtest. Ratio traits are generally not recommended in animal breeding (Zetouni *et al*., 2017). For instance, the heritability of a ratio trait cannot be used to predict the genetic change for the ratio trait (Gunsett, 1987). Therefore, we recommend using the absolute $U_{crit}$ and to fit either Wtest or SLtest as a covariate in a model when estimating heritability. Such an analysis shows the existence of heritable variation in $U_{crit}$ beyond body size.

The less than unity genetic correlation between absolute $U_{crit}$ and Wtest indicates the presence of genetic variation in $U_{crit}$, independently of Wtest. A genetic correlation of unity between two traits means that the two traits are controlled by the same genes while a genetic correlation of less than unity indicates that there are additional genes that are not common for the two traits and only control one of the two traits. The negative $r_g$ between absolute $U_{crit}$ and HW, and between $U_{crit}$ and DGC, clearly indicates that selection for high harvest weight will favour faster growing animals with lower $U_{crit}$. Whether this is desirable needs to be determined. One can speculate that under conditions of hypoxia, as frequently encountered in non-aerated ponds or ponds with algal blooms, smaller, more active fish will have a higher chance of survival. In optimal management conditions, however, growth rate can be further increased by including $U_{crit}$ at testing in the breeding goal, next to harvest weight. Fish with higher $U_{crit}$ may also be more resilient: swimming exercise improves physiological fitness; cardiovascular and respiratory performance, and increases mitochondrial densities and muscle tissue capillarization (Palstra and Planas, 2011). Also the immune system capacity appears to be linked to swimming performance as Castro *et al*. (2013) found 21 virus-responsive genes with significantly higher transcript abundance in phenotypically poor swimmers as compared to good swimmers in Atlantic salmon. In conclusion, including absolute $U_{crit}$ in a breeding goal in addition to HW and DGC could be beneficial if the aim is to select for fitter fish, especially in environments where oxygen is limiting. Absolute $U_{crit}$ can be measured at an early stage on the selection candidates themselves, high throughput and non-invasively although size of the tested fish may be restricted due to difficulty in reaching sufficiently high flow speed. However, selection on $U_{crit}$ with 10% selection intensity from the highest

value of $U_{crit}$ could lead to 19% reduction in mean harvest weight of the offspring, compared to direct selection on harvest weigh. In practice, we recommend a two stage selection scheme, where selection in the first stage is on retaining 90% of the fittest fish in terms of $U_{crit}$, followed by a second stage selection on harvest weigh. This study showed for the first time the existence of significant additive genetic variance for critical swimming speed in Nile tilapia. Favourable $r_g$ between $U_{crit}$ and traits such as Wtest, SLtest, Htest and SAtest early in life were found. The main finding demonstrated a negative $r_g$ between $U_{crit}$ and HW later in life, and between $U_{crit}$ and DGC later in life. Including $U_{crit}$ in the breeding goal may help to improve resilience of Nile tilapia.

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## Appendix A

**Table 2.7**: Mean water velocity (cm $s^{-1}$) and standard deviation at different each propeller speed level.

| Propeller speed level | Mean velocity cm $s^{-1}$ | Standard deviation |
|---|---|---|
| 2 | 18.60 | 1.25 |
| 3 | 31.32 | 1.63 |
| 4 | 41.83 | 1.39 |
| 5 | 51.97 | 1.70 |
| 6 | 59.99 | 2.48 |
| 7 | 66.60 | 3.65 |
| 8 | 72.90 | 4.67 |
| 9 | 77.62 | 5.82 |
| 10 | 80.84 | 7.89 |

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CHAPTER 6

# General discussion

## 6.1 Nile tilapia production and challenges

Animal source protein demand is increasing all over the world due to human population growth and economic development (Delgado, 2003; Popkin *et al*., 2012). Animal source protein intake is still relatively low in developing countries but the rate of increase is much higher compared to the trends in developed countries (Delgado, 2003). Nile tilapia aquaculture can contribute to meet the increasing demand in animal source protein. Nile tilapia is a good source of cheap and affordable protein and can be grown on lower grade feed ingredients that are not suitable for human consumption. Therefore, improving Nile tilapia aquaculture production can play a great role in food and nutrition security and poverty alleviation, thereby contributing to the United Nations sustainable development goal number 2 which is achieving "zero hunger" (United Nations General Assembly, 2015).

The production of Nile tilapia in the world has increased substantially in recent years (FAO, 2020). Genetic improvement and nutrition are among the main contributors for the increased production. The genetically improved farmed tilapia (GIFT) breeding program is an important non-commercial breeding program that achieved substantial genetic gains for harvest weight (Bentsen *et al*., 2017). The GIFT strain has been distributed worldwide (Agha *et al*., 2018). However, there are still large differences in productivity among smallholder Nile tilapia farms despite the use of genetically improved strains such as the GIFT strain. This so-called yield gap is caused by differences in growth, feed conversion ratio (FCR) and survival, the key traits that affect production efficiency (Kankainen *et al*., 2012; de Verdal *et al*., 2018).



Growth and FCR are mainly affected by temperature, dissolved oxygen (DO), pH and crude protein content of feeds (Chapter 2). Under pond aquaculture, it is hard to influence water temperature but DO, pH, crude protein content of feeds, stocking weight and stocking density, can be optimized by management. DO is generally managed by aerating ponds. Not aerating leads to large fluctuations in DO throughout the day. In the GxE experiment (Chapter 3), day-time DO concentrations in non-aerated pond during 219 days of the grow-out period of Nile tilapia were above 3 mg/L. However, the DO concentrations started to decline after sunset and dropped below one mg/L just before sunrise (Fig. 6.1). The negative effect of recurrent hypoxia (DO < 3 mg/L) on productivity of Nile tilapia increased over time during the grow-out period (Table 6.1). The recurrent hypoxia reduced the mean body weight and survival in the non-aerated pond compared to the

aerated pond. The FCR (feed required per kilogram biomass) was higher in the non-aerated pond than the aerated pond in all the grow-out periods. Nile tilapia requires above 5 mg/L DO concentration for optimal performance (Tran-Duy *et al.*, 2012), and the recurrent hypoxic production environment is therefore challenging and probably stressful to Nile tilapia since such conditions negatively affect growth and welfare. The increased FCR in the non-aerated pond could also be due to lower feed assimilation and/or suppressed feed intake and feed waste. From Nile tilapia behaviour monitoring, I personally observed that Nile tilapia in the non-aerated pond stopped feeding during early morning while Nile tilapia in the aerated pond were active and with good appetite.

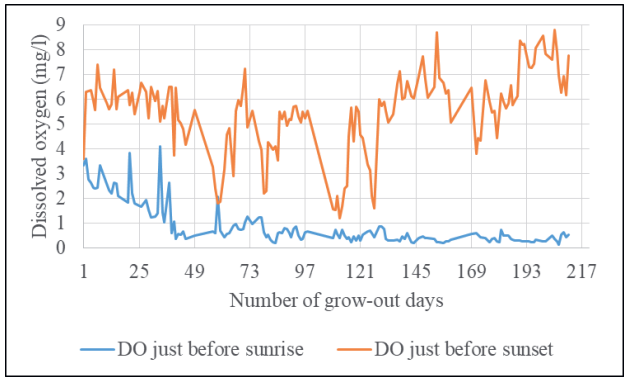


Figure 6.1 Morning and sunset dissolved oxygen (DO) concentrations (mg/l) in the non-aerated pond during grow-out period.

Table 6.1 Average body weight, survival percentage and feed conversion ratio (FCR) (results not shown in previous chapters) during four different grow-out periods of 219 grow-out days for Nile tilapia stocked in aerated and non-aerated ponds.

| Grow-out period | Aerated pond | | | Non-aerated pond | | |
|---|---|---|---|---|---|---|
| | Average body weight (g) | Survival (%) | FCR | Average body weight (g) | Survival (%) | FCR |
| First (51 days) | 25 | 76 | 1.21 | 25 | 79 | 1.24 |
| Second (42 days) | 160 | 90 | 2.32 | 143 | 85 | 2.57 |
| Third (56 days) | 292 | 94 | 1.77 | 265 | 97 | 2.15 |
| Fourth (48 days) | 535 | 91 | 1.71 | 425 | 88 | 4.05 |
| Total | 535 | 64 | 1.73 | 425 | 57 | 2.31 |

DO concentration is affected by algal photosynthesis, respiration of aquatic organisms, and the diffusion of atmospheric oxygen into the water (Abdel-Tawwab *et al*., 2019). Respiration of fish, algae, and bacteria is temperature dependent. In the future, global warming is also expected to raise the water temperature, thereby increase biological DO demand and further decreases DO concentration in aquatic habitats. (Lennox *et al*., 2019). Therefore, recurrent hypoxia is expected to become a serious problem in non-aerated pond Nile tilapia aquaculture in the near future. Aerating ponds for a few hours during night-time could alleviate the problem of recurrent hypoxia. However, aerator equipment is expensive and costly to repair for smallholder Nile tilapia farmers and/or smallholder farmers may have no access to cheap electricity or stable electricity supply. Therefore, I recommend that Nile tilapia breeding programs should also consider using selective breeding to improving Nile tilapia performance under recurrent hypoxia conditions. More specifically, Nile tilapia breeding programs should look for resilience traits to improve Nile tilapia growth and survival and reduce the impact of a hypoxic environment.

Resilience is defined as the capacity of an animal to be minimally affected by perturbations or to quickly recover to the state it had before the perturbation (Colditz and Hine, 2016). The Log-transformed variance of deviances (LnVar) of body weight is one of the indicators of resilience. LnVar in Nile tilapia is a heritable trait (Chapter 4). In dairy cows and pigs, lower LnVar was indicative of better health and survival. Low LnVar of milk yield of dairy cows has been reported to be favorably correlated with traits such as health, ketosis, fertility and longevity traits (Elgersma *et al*., 2018; Poppe *et al*., 2021). Mulder *et al*. (2015) found a favorable genetic correlation between the residual variance of piglet birth weight, an indicator of resilience, and survival at birth. There is a variation among individual fish growth rate in a hypoxic environment which could be associated with their specific coping styles (Damsgård *et al*., 2019). Understanding the growth pattern of fish from stocking to harvest is important for monitoring welfare (Huntingford *et al*., 2006). LnVar can be used to quantify such disturbances during the grow-out period.

The critical swimming performance ($U_{crit}$) of Nile tilapia is another trait potentially associated with resilience. Forced exercise, “Swimming”, promotes fish welfare through its positive effect on muscular-skeletal development, osmoregulation, and disease resistance (Huntingford and Kadri, 2013). The ability to swim can be measured in a treadmill-like swimming carrousel where fish swim against

increasing currents until they fatigue ($U_{crit}$). Nile tilapia with higher $U_{crit}$ are expected to have higher cardio-vascular fitness and may cope with recurrent hypoxia better than fish with lower $U_{crit}$.

## 6.2 Breeding goal

A breeding goal described the desired direction of change for a set of traits. In almost all Nile tilapia breeding programs, the breeding goal is to increase harvest weight. This goal is achieved by selecting fish with the highest estimated breeding values (EBVs) to be parents of the next generation. Nile tilapia tolerant to recurrent hypoxia and less disturbed by stressors could have a higher survival rate and lower FCR under smallholder farms. Therefore, defining a breeding goal for selective breeding of Nile tilapia that can cope under smallholder farms, are less affected by stressors and perform better, is important.

Nile tilapia farm profit depends on growth, FCR and survival of the fish. Individual fish feed intake in ponds is difficult to measure, which makes the selection effort for low FCR a challenging process. FCR is also a ratio trait and selecting for a ratio is less effective than a linear index (Gunsett, 1984). Improving survival is also difficult because of the confounding effect of many factors causing mortality; consequently the heritability of survival is low. Mortality of Nile tilapia can be caused by disease causing organisms such as bacteria, viruses, fungi and parasites, and stressors such as low or fluctuating dissolved oxygen. Stress due to different factors and poor water quality also contributes to mortality of Nile tilapia. Therefore, Nile tilapia breeding programs should consider additional economic and/or non-economic traits related to FCR and survival.

## 6.3 Traits

Accurate trait measurement is key to genetic improvement. In Chapter 3, we weighed each individual fish using a weighing scale, analysed body length and height from the photographs using digital image analysis and calculated surface area of fish. Harvest weight and surface area were strongly genetically correlated (0.99). The strong genetic correlation shows that body weight can be improved by selecting based on surface area. The coefficient of variations for harvest weight was 1.4 to .5 times larger than the coefficient of variations for surface area. Thus, surface area using digital image is more accurate than body weight measurement using weighing scale in the field. Digital image analysis requires less time and is storable (Blonk *et al.*, 2010). It is not also prone to recording errors and less stressful for the fish compared to the manual method. Therefore, it is

recommended that Nile tilapia breeding programs should consider using fish surface area instead of body weight.

Resilience indicators such as LnVar and $U_{crit}$ are important traits to consider for improving FCR and survival in Nile tilapia. LnVar could capture the response of Nile tilapia growth to all the disturbances while $U_{crit}$ reflect the cardio-vascular fitness of Nile tilapia. The desired direction of selection for LnVar is downward (negative), reducing LnVar by selecting animals with low EBVs for LnVar while for $U_{crit}$ the desired direction of selection is upward (positive).

Results from Chapter 4 showed that LnVar based on five time-points individual body weight measurements in Nile tilapia was moderately heritable and can be improved by selective breeding. Breeding programs measure body weight at stocking and harvest. LnVar based on five measurements requires three additional measurements in between stocking and harvest. We also estimated genetic parameters of LnVar based on three time-points body weight measurements, (results not presented in Chapter 4). These are summarized in Table 6.2. LnVar based on three time-point body weight measurements (LnVar-3) requires only one extra measurement in addition to stocking and harvest body weight measurements. LnVar-3 gave similar heritability estimates and higher genetic coefficient of variations (GCV) compared to LnVar based on five body weight measurements (LnVar-5). The heritability estimates for LnVar-3 in the aerated pond was 0.12±0.04 and in the non-aerated pond was 0.15±0.05. The GCVs based on three time-point body weight measurements were 48.0% in the aerated pond and 56.8% in the non-aerated pond, while the GCVs based on five measurements were 32.2% in the aerated pond and 34.4% in the non-aerated pond. The genetic correlations between LnVar based on three body weight measurements and survival days in the aerated (-0.27±0.34) and in the non-aerated (-0.32±0.39) ponds suggest that fish with higher LnVar have lower survival days in both ponds. Selecting for lower LnVar could therefore improve survival and health of animals. Animals with lower Lnvar showed more uniform growth. More uniform growth and better survival will improve productivity by improving the accuracy of biomass estimation, feeding management and survival percentage.



The results indicated that three repeated body weight measurements could give a similar result to five repeated body weight measurements. Therefore, it is recommended that breeding programs should consider using LnVar based on three

repeated body weight measurements over time, at least until automated digital imaging is available.

More number of records per individual over time would improve genetic gain in LnVar by increasing the accuracy of selection. In the future, weekly or biweekly individual records can be obtained using a computer vision system (CVS) with less labour and without stressing the fish. Hufschmied *et al*. (2011) collected images of swimming fish using a camera of an automatic grading machine and estimated body weight using a linear regression method. Recently Fernandes *et al*. (2020) developed CVS for autonomous measurement of Nile tilapia. The method avoids handling of the fish and the related stress caused due to handling. As the technology is advancing in the future, it would enable us to collect more accurate and repeated measurements per individual fish over-time without stressing the fish. Genetic gain ($\Delta G$) per generation is a function of selection intensity (i), accuracy of selection (r), and genetic standard deviation ($\sigma_a$), generation interval (L) $\Delta G = (i * r * \sigma_a)/L$ (Falconer and Mackay, 1996). More records per individual fish over time increases selection accuracy and expected genetic gain.

Table 6.2 Additive genetic variances ($\sigma_a^2$), phenotypic variances ($\sigma_P^2$), heritabilities ($h^2$) and genetic coefficient of variance (GCV) for log-transformed variance of deviances based on three time points body weight measurements (LnVar-3) and based on five time points body weight measurements (LnVar-5) within aerated and non-aerated ponds.

| Trait | Environment | $\boldsymbol{\sigma_a^2}$ | $\boldsymbol{\sigma_P^2}$ | $\boldsymbol{h^2}$ | GCV |
|---|---|---|---|---|---|
| LnVar-3 | Aerated | 0.230 | 1.872 | 0.12±0.04 | 48.0 |
| | Non-aerated | 0.332 | 2.139 | 0.15±0.05 | 56.8 |
| LnVar-5 | Aerated | 0.091 | 0.907 | 0.10±0.05 | 30.2 |
| | Non-aerated | 0.118 | 0.988 | 0.12±0.05 | 34.4 |

In Chapter 5, we showed that $U_{crit}$ is a moderately heritable trait (0.41 to 0.48). $U_{crit}$ early in life was unfavourably correlated with harvest weight (-0.21) and growth (-0.63), however, these genetic correlations between $U_{crit}$ and the production traits come with large standard errors and the true correlation with HW is currently unclear. Swimming performance test is less stressful for the fish than body weight measurement and it requires a single test at a fingerling stage. However, swimming performance test is time consuming, requires similar size fish from different families to test together and requires well-trained technicians. In addition, the maximum number of fish that can be swim performance tested per swim flume per day is around 50. These limitations make testing large numbers of Nile tilapia a challenge.

$U_{crit}$ measurements and correlations come with large standard errors and are more time consuming than LnVar. Although the handling and measurements are relatively more stressful to the fish, LnVar can be done in a few days. Therefore, it is recommended that breeding programs should use LnVar.

## 6.4 Optimization of breeding program for GIFT

The GIFT strain is selected under optimal dissolved oxygen, while smallholder production ponds who are producing GIFT are hypoxic during the night. One can expect less than unity genetic correlations between the same traits measured in the two environments. GxE interaction for a trait of interest is quantified by estimating the genetic correlation between traits recorded on full sibs and half sibs in different environments and regarding the trait as different traits (Falconer, 1952). We found genetic correlations of 0.81 for harvest weight and 0.78 for thermal growth coefficient (Chapter 3) and 0.80 for LnVar (Chapter 4) between the aerated and non-aerated environments. A less than unity genetic correlation between the two environments indicates the presence of GxE interaction. To consider GxE interactions biologically important, Robertson (1959) suggested that the genetic correlation between the two environments needs to be less than 0.8. Mulder *et al.* (2006) recommended the use of separate breeding programs for different environments when the genetic correlation is less than 0.61. However, establishing and running separate breeding programs is costly. Therefore, the economic benefit of establishing separate breeding programs should be carefully weighed to justify investments in separate breeding programs. In the presence of GxE, half-sib information from production environments could help to minimize the reduction in genetic gain in correlated traits (Brascamp *et al.*, 1985; Mulder and Bijma, 2005). Our genetic correlation estimates are ≥ 0.78. Therefore, a single Nile tilapia breeding program for different environments and the use of sib information from non-aerated ponds would be recommended.



In the future, genomic selection with a reference population with genotype and phenotype from the production environment could increase response in the production environment (Mulder, 2016). Barría *et al*. (2021) found comparable accuracies using a minimum of 5k SNPs to that of high-density markers with much lower cost. The cheaper low-density SNP markers with comparable accuracies could be a good option for setting up genomic selection programs to improve productivity in a non-aerated production environment. It could also help to avoid separate full sib family rearing.

I made a comparison of correlated selection responses (CR) in a non-aerated pond for breeding goals that included only HW with different selection indices, a breeding goal and index that included HW and LnVar and a breeding goal and index that included HW and $U_{crit}$ (summarized in Table 6.3). In all cases, sib performance from a non-aerated and aerated pond was used as information sources. The genetic gains for different breeding goals following discrete one-stage selection simulation are predicted using Sel*A*ction (Rutten et al., 2002). For the simulation, genetic parameters from Chapters 4 and 5 were used, and 50 males and 100 females mated each discrete generation, producing 25 male and 25 female offspring per female. Each generation 2 % of males and 4 % of females were selected to produce the next generation.

The CR response in HW was 57.8 g when the breeding goal and index information included only HW. Including additional traits in the breeding goal and index could reduce the CR in HW. Therefore, five percent was taken as a maximum acceptable reduction in response for HW. In a breeding goal that included only HW, including either LnVar or $U_{crit}$ in addition to HW in the index resulted in a higher CR in HW but the CR in LnVar and $U_{crit}$ was in an undesired direction. However, including both HW and LnVar or HW and $U_{crit}$ in the breeding goal and selection index resulted in improvement in HW and LnVar or HW and $U_{crit}$. In a breeding goal that included HW and LnVar using BLUP selection plus sib information from non-aerated pond, a considerable weight had to be placed on selection for low LnVar (Fig. 6.2). Placing a high weight on selection for low LnVar lead to a reduction in CR in HW. The maximum CR in LnVar was -0.111 using 55 times more weight on LnVar compared to HW. Note that harvest weight has a much higher phenotypic variance than LnVar. Not using sib information from non-aerated pond reduced CR by 4 to 15 percent in HW. In a breeding goal that included HW and $U_{crit}$, placing more weight on $U_{crit}$ instead of equal weight resulted in higher CR in $U_{crit}$ with only 5% CR reduction in HW from 57.8 g. Therefore, breeding programs could improve both HW and LnVar, and HW and $U_{crit}$ by including LnVar or $U_{crit}$ in their breeding goal with an appropriate weight relative to HW.

In all cases, the rate of inbreeding per generation was high. Use of BLUP in estimating breeding values (EBVs) emphasizes the selection of relatives, which leads to inbreeding. The 50% of Mendelian sampling variance that exists within a family is not exploited (Fjalestad, 2005). In practice, the optimum contribution method is used for controlling inbreeding in aquaculture (Skaarud *et al*., 2011).

Table 6.3 Correlated selection responses in the non-aerated pond for selection in the aerated pond for different breeding gaols trait(s) and indices using best linear unbiased prediction (BLUP) plus sib information from the non-aerated pond. (See Table 6.4 for the input parameters)

| Breeding goal | Index | Correlated selection response in non-aerated pond | | |
|---|---|---|---|---|
| | | HW | LnVar | $U_{crit}$ |
| H = HW† | HW | 57.8 | - | - |
| H = HW | HW and LnVar | 58.1 | 0.006 | - |
| H = HW | HW and $U_{crit}$ | 61.9 | - | -0.874 |
| H=1*HW - 55*LnVar | HW and LnVar | 54.9 | -0.111 | - |
| H = 1*HW + 9*$U_{crit}$ | HW and $U_{crit}$ | 54.6 | - | 0.759 |

For all the traits in selection index I used Own performance, BLUP, 24 full sibs and 25 half sibs records from non-aerated pond and 25 full sibs and 25 half sibs records from non-aerated pond.

†Used as a reference to compare the correlated selection response of the other breeding goals and indices.

**Table 6.4** Parameters used in SelAction. Heritabilities and phenotypic variances in brackets (diagonal), genetic correlations (above diagonal) and phenotypic correlations (below diagonal).

| | HW-A | LnVar-A | $U_{crit}$-A* | HW-NA | LnVar-NA | $U_{crit}$-NA* |
|---|---|---|---|---|---|---|
| HW-A | 0.23 (37274) | 0.36 | 0.38 | 0.80 | -0.03 | 0.38 |
| LnVar-A | 0.10 | 0.10 (0.907) | | 0.41 | 0.80 | |
| $U_{crit}$-A | -0.04 | | 0.41 (16.09) | -0.21 | 0.00 | 0.999 |
| HW-NA | | | -0.04 | 0.18 (15148) | -0.01 | -0.21 |
| LnVar-NA | | | | 0.04 | 0.12 (0.988) | |
| $U_{crit}$-NA | -0.04 | | 0.999 | -0.04 | 0 | 0.41 (16.09) |

*The two traits are the same traits since they were measured before the fish is stocked in the grow-out ponds.
The LnVar and $U_{crit}$ were not measured on the same individual and correlations between were not estimated.

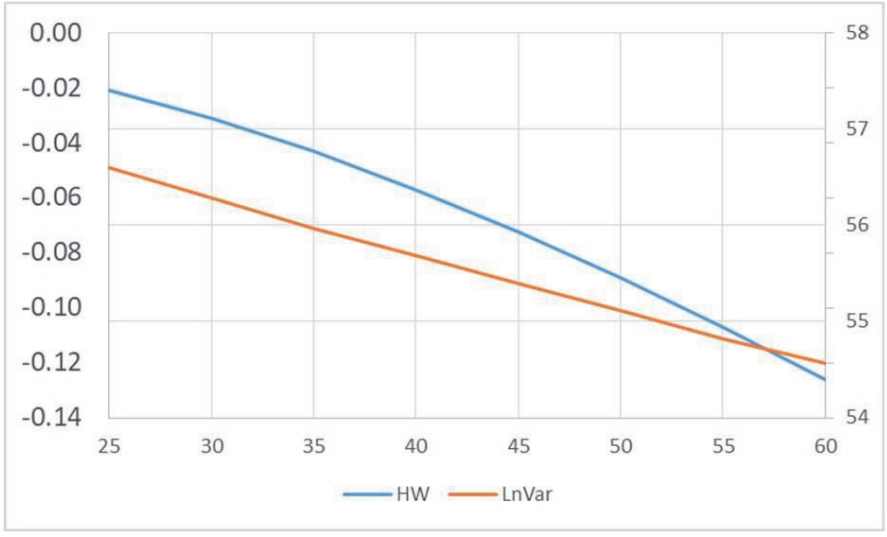


Figure 6.2 Genetic gains for harvest body weight (HW) and log-transformed variance of deviances (LnVar) (in trait units) for different relative weights. The breeding goal (H) H=1*HW +weight*LnVar, the relative weight for HW was kept 1 and the relative weight for LnVar was negative and increased until the response to HW equals to 54.9 g. See Table 6.4 for the parameters used.

## 6.5 Implementation of Nile tilapia family production and rearing

Classical Nile tilapia genetic improvement programs often consist of full sib and half sib family groups in a nucleus and multipliers at a different station with the objective to multiply and disseminate genetically improved Nile tilapia. In Malaysia, WorldFish does the selective breeding work of the GIFT strain, while Malaysia's Department of Fisheries does the multiplication and dissemination.

In the GIFT breeding program, the families are traditionally produced by stocking one male and one female in a mating hapa (WorldFish Center, 2004). Eggs from a successfully mated females' mouth are collected and incubated separately. After yolk sac absorption, the full sib families are reared separately in hapas until they reach tagging size (about 5 g). In the GIFT breeding program, the full sib family group production takes up to three months (Trọng *et al*., 2013), and this long family

production period increases the time full-sib family groups spend separately. The separate family rearing introduces common environmental effects to full sib groups. For example, DO concentrations differences among hapas could affect growth of juvenile Nile tilapia (Charo-Karisa *et al*., 2006). Estimated common environmental variance as a proportion of phenotypic variance for different traits ranged from 0.02 to 0.23 (Rutten *et al*., 2005; Bentsen *et al*., 2012; Gjerde *et al*., 2012). The long time required to produce family groups is one of the main hurdles in Nile tilapia genetic improvement programs.

Common environmental effects exist in Nile tilapia breeding programs, and unaccounted common environmental variation impairs the efficiency of family selection (Fjalestad, 2005). Not accounting for significant common environmental effects results in upward biased selection accuracies. In many cases, this classical family production method produces problems when estimating genetic parameters: models do not converge when fitting common environmental variance, or the common environmental variance absorbs all the (co)variances when the models do converge (Maluwa *et al*., 2006; Trọng *et al*., 2013; Omasaki *et al*., 2016). Standardizing the nursery environment for all families, shortening the duration of the separate full sib family production period and pooling together as early as possible after tagging could minimize the common environmental variance (Fjalestad, 2005).

Creating sufficient full sib and half sib family relationships is crucial to disentangle additive genetic and common environmental effects and help to overcome the problem that arises from the common environmental effects (Gjerde, 2005). Recently, Trọng (2013) recommended using one male to five females mating design to produce more full sib and half sib family groups in a short period. The design requires fewer mating tanks/hapas and enables the production of a sufficient number of family groups in about 28 days compared to up to three months using a mating design with one male and only one/two females. This method also reduces the age difference among families and the separate family rearing period.

In our study, the family groups were mass produced in four hapas in 15 days using eighteen males and fifty females per mating hapa, 72 males and 200 females in total, and nursed together in the same hapa until tagging (Figure 6.3). This resulted in non-significant ("0") common environmental variance estimates (Chapter 3). Genomic relationships were used to construct the relationship matrix. In Nile tilapia, a natural mating pattern could range from single pair to promiscuous

mating (Fessehaye *et al*., 2006) that could result in a mixed nested/factorial mating design. Mixed nested/factorial mating design enables disentangling additive and common environmental effects and provides accurate estimates of parameters. However, in mass production, the mating is uncontrolled and inbreeding could be an issue (Fessehaye *et al*., 2009). This can be minimized by minimizing the co-ancestry of breeders stocked in the same mating hapa using genomic relationships. Therefore, it is recommended to use multiple males and females to produce families and genomic relationships to estimate genetic parameters.

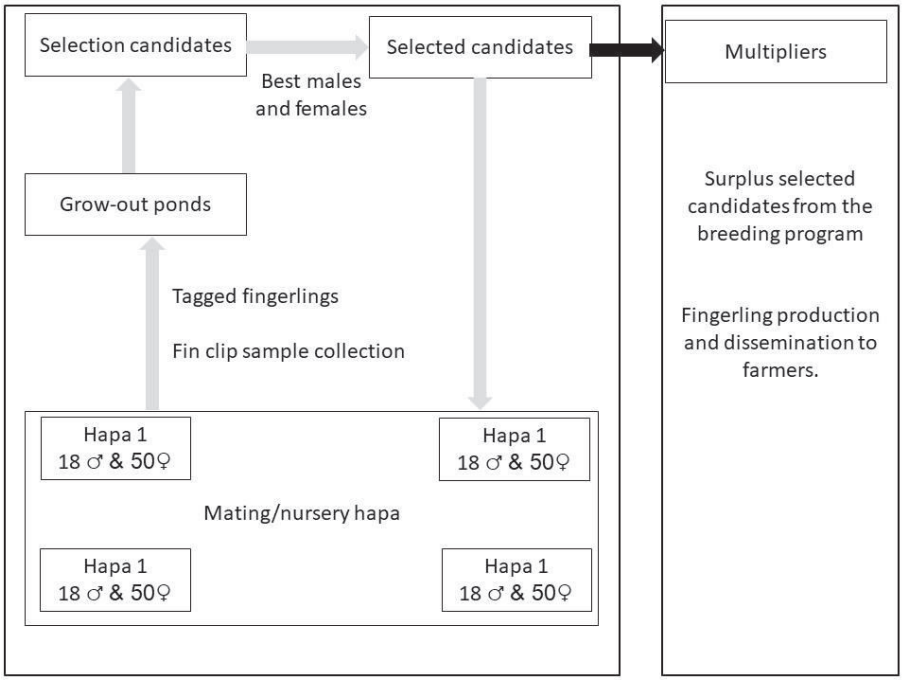


Figure 6.3 Main elements in mass production of families of Nile tilapia for selective breeding.

**6.1. Conclusion**

This research has demonstrated the major yield gap factors and the opportunities to contribute to close the yield gap under smallholder Nile tilapia farmers. Nile tilapia is produced mainly in developing countries where food and nutrition security is a great concern. Improving the production efficiency of smallholder Nile tilapia farms could substantially contribute to food and nutrition security and livelihood of households in developing countries. Currently, Nile tilapia breeding programs mainly select for improved harvest weight. An accurate estimate of genetic parameters for production and resilience traits are prerequisite inputs for designing breeding programs that can contribute to reduce the yield gap under

smallholder Nile tilapia farms. A comparison of selection response of different breeding goals and indices showed that including Log-transformed of variance deviances both in the breeding goal and index can lead to a reduction in variation, and more uniform, individual growth trajectories over time. Including swimming performance both in the breeding goal and index with harvest weight can lead to an improvement in swimming performance and fitness of Nile tilapia. Breeding programs could also benefit from the use of sib information from non-aerated ponds to increase selection response for harvest weight, Log-transformed variance of deviations and swimming performance. Nile tilapia breeding programs can shorten prolonged family production time by using multiple males and females.

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# Summary

## Summary

Smallholder Nile tilapia farms underperform in terms of feed efficiency, despite the use of genetically improved strains of tilapia such as GIFT. Big differences in productivity among many smallholder tilapia farms are observed leading to a yield gap between the best performing and low performing farms. Therefore, the aim of this thesis was to optimise the breeding program of Nile tilapia for a smallholder production system, thereby contributing to closing the yield gap, the difference between the best performers and lower ones, is the major concern for small- and medium-scale Nile tilapia farms. The specific objectives were: i) to quantify the effects of the most likely environmental and management factors on FCR, mortality and growth of Nile tilapia, ii) to investigate the presence of genotype by environment interaction between selection and production environments, iii) to estimate genetic parameters for resilience and iv) to estimate genetic parameters for swimming performance of Nile tilapia (novel trait) and to estimate the genetic correlation between swimming performance and production traits in aerated and non-aerated ponds.

The yield gap is affected by differences in growth rate and feed conversion ratio (FCR). FCR at the farm level is strongly influenced by survival of fish. Identification of the factors that lead to the yield gap is important before any intervention to close the yield gap. In **chapter II**, we conducted a systematic literature review of two databases (ASFA and CAB-Abstracts) to quantify the effects of the most likely environmental factors on FCR, mortality and growth. Results showed that increasing stocking weight (SW) significantly improved both FCR and survival. Temperature had the largest effect on FCR followed by dissolved oxygen (DO), pH and CP. DO had the largest effect on TGC followed by crude protein (CP) and pH. This study confirms that the optimal rearing temperature for Nile tilapia is between 27 and 32℃. Improving management to optimize DO ($> 5 \mathrm{mg\ L^{-1}}$), stocking density ($3 - 5\ \mathrm{fish\ m^{-2}}$), SW (>10g) and CP ($25 - 30\%$) will improve performance and survival in small- and medium-scale tilapia farming. However, it is hard to influence temperature in ponds and cages while DO is largely influenced by aeration. Since many small- and medium-sized farms do not have aeration, these major tilapia farming systems could benefit from genetically improved strains selected for resilience to highly fluctuating diurnal temperature and DO levels.

Nile tilapia has been selectively bred under optimal dissolved oxygen environment but most smallholder production still takes place in non-aerated ponds which have large diurnal oxygen fluctuations. In the presence of environmental differences

between production and selection environment, genetic gains achieved in selection environment may not be fully realized in production environment. Therefore, knowledge of GxE interaction is important in designing and optimizing breeding programs. In **chapter III**, genetic parameters for harvest weight (HW), thermal growth coefficient (TGC), surface area (SA) and body shape, expressed as ellipticity (Ec) and their GxE interactions between aerated and non-aerated ponds were estimated and the impact of (non-)aeration on genetic parameters were investigated. The experimental fish were mass-produced using natural group spawning and nursed in four $30m^2$ hapas. Of the stocked fish, 2063 were genotyped-by-sequencing (GBS). A genomic relationship matrix was built using 11,929 SNPs to estimate G-BLUP parameters. No-aeration had a strong negative impact on mean HW, genetic variance and genetic coefficient of variation. Substantial heritabilities (0.14-0.45) were found for HW, TGC, SA and Ec and low heritabilities (0.03–0.04) for survival in aerated and non-aerated ponds. In both ponds, the environmental effect common to full sibs was not significant. Genetic coefficients of variation were 20–23% lower and heritabilities were 19–25% lower in the non-aerated pond compared to the aerated pond, for HW, TGC and survival. Genetic correlations between ponds for HW, standard length, height, SA and TGC were 0.81, 0.80, 0.74, 0.78 and 0.78, respectively. In summary, some GxE interaction between aerated and non-aerated ponds was found and no-aeration decreased genetic coefficients of variation and heritabilities compared to aerated ponds. Breeding programs are recommended to use half sib information from non-aerated farms or to set up a reference population for genomic selection in a non-aerated environment either on-station or in farms.

Resilience is an important trait in Nile tilapia. Log-transformed variance of deviations (LnVar) one of the indicators of resilience. In chapter IV, we estimated genetic parameters for resilience in Nile tilapia, using LnVar of body weight measured five times during grow-out in either an aerated or a non-aerated pond. The heritability for LnVar was 0.10 in aerated pond and 0.12 in the non-aerated pond. In aerated ponds the genetic correlation ($r_g$) of LnVar with harvest weight (HW) was 0.36±0.26, and with thermal growth coefficient (TGC) it was 0.47±0.21. In the non-aerated pond, the $r_g$ with HW and TGC were close to zero (-0.01±0.29 and-0.08±0.22). The genetic correlation for LnVar between both environments was 0.80. These estimates suggest that selection for HW or TGC in aerated ponds will increase LnVar in both environments. Increased LnVar may decrease resilience and this will be detrimental to performance. Selecting for more resilient fish would lead to more constant growth rates, which makes biomass estimation more accurate

and could therefore result in more optimal feeding regimes and less feed waste. This would have a favourable effect on the feed efficiency in production units and on the environmental impact of fish farming. To improve resilience together with growth we recommend that fish breeding programs collect repeated records on body weight, preferably in challenging environments.

Critical swimming speed ($U_{crit}$) another indicator of resilience. We hypothesize that Nile tilapia with high oxygen uptake efficiency ($O_2$UE) may perform better under these conditions than Nile tilapia with low $O_2$UE. Critical swimming speed ($U_{crit}$) is a potential indicator for $O_2$UE. In chapter V, we estimated variance components for $U_{crit}$ and fish size at swim testing, and genetic correlations ($r_g$) between $U_{crit}$ with harvest weight (HW) and daily growth coefficient (DGC) after grow-out in a non-aerated pond. Substantial heritability was found for absolute $U_{crit}$ (in $ms^{-1}$; 0.48). The estimated $r_g$ between absolute $U_{crit}$ and fish size at testing were all strong and positive (range 0.72 - 0.83). The estimated $r_g$ between absolute $U_{crit}$ and HW, and absolute $U_{crit}$ and DGC were -0.21 and -0.55 respectively, indicating that fish with higher absolute $U_{crit}$ had lower growth in the non-aerated pond as compared to fish with lower absolute $U_{crit}$. These results suggest a juvenile trade-off between swimming and growth performance where fish with high $U_{crit}$ early in life show slower growth later under conditions of limited oxygen availability. We conclude that $U_{crit}$ in Nile tilapia is heritable and can be used to predict growth performance.

In Chapter VI, I discussed smallholder Nile tilapia production challenges, different family production methods and selection responses to different breeding goals and selection indices.

The results from deterministic simulation showed that HW and LnVar , HW and $U_{crit}$ in a non-aerated pond can be improved simultaneously by selective breeding in an aerated pond by placing the right relative weight on LnVar or $U_{crit}$.

# Curriculum Vitae

## About the author

Samuel Bekele Mengistu was born on February 07, 1977 in Ethiopia. He studied for his diploma in General Agriculture at Hawassa University and graduated with distinction in 1997. After graduation Samuel joined Ministry of Agriculture served in different capacities until he started his study for BSc degree. In 2005, he earned his BSc degree with distinction in Animal and Range Sciences from Hawassa University. After his graduation, he joined Hawassa University and served as a graduate assistant until he left to Norway for his MSc degree study. In 2010, he graduated from Norwegian University of Life Sciences (UMB) with MSc in Aquaculture. His thesis was on genetic and phenotypic parameters for body weight and fillet traits of Nile tilapia. After earning his MSc degree, he resumed his work at Hawassa University as a Lecturer and served until 2016. In 2016, he started his PhD study at Animal Breeding and Genomics group, Wageningen University and Research. All his PhD study experiments were done in Malaysia WorldFish research center and the project was funded by The Koepon Foundation and WorldFish. This thesis is the result of his PhD study. Currently, Samuel is working as a Lecturer at Hawassa University Ethiopia.

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## Peer reviewed publications

**Mengistu, S. B**., Mulder, H. A., Bastiaansen, J. W. M., Benzie, J. A. H., Khaw, H. L., Trinh, T. Q., Komen, H. 2022. Fluctuations in growth are heritable and a potential indicator of resilience in Nile tilapia (*Oreochromis niloticus*). *Aquaculture,* 560, 738481.

Yu, X., **Mengistu, S. B.**, Mulder, H. A., Palstra, A. P., Benzie, J. A. H., Trinh, T. Q., Groenen, M. A. M., Komen, H., Megens, H.-J. 2022. Quantitative trait loci controlling swimming performance and their effect on growth in Nile tilapia (Oreochromis niloticus). *Aquaculture,* 560, 738522.

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Yu, X., Megens, H.-J., **Mengistu, S. B.**, Bastiaansen, J. W. M., Mulder, H. A., Benzie, J. A. H., Groenen, M. A. M., Komen, H. 2021. Genome-wide association analysis of adaptation to oxygen stress in Nile tilapia (Oreochromis niloticus). *BMC Genomics,* 22(1)**,** pp 426.

**Mengistu, S. B.**, Mulder, H. A., Benzie, J. A. H., Khaw, H. L., Megens, H.-J., Trinh, T. Q., Komen, H. 2020. Genotype by environment interaction between aerated and non-aerated ponds and the impact of aeration on genetic parameters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture,* 529, 735704.

**Mengistu, S. B.**, Mulder, H. A., Benzie, J. A. H., Komen, H. 2020. A systematic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia (Oreochromis niloticus). *Rev Aquacult,* 12(2)**,** 524-541.

Gjerde, B., **Mengistu, S. B.**, Ødegård, J., Johansen, H., Altamirano, D. S.: 2012. Quantitative genetics of body weight, fillet weight and fillet yield in Nile tilapia (*Oreochromis niloticus*). *Aquaculture,* 342-343, 117-124.

## Contributions to conferences

Yu, X., **Mengistu, S. B.**, Mulder, H. A., Palstra, A. P., Benzie, J. A. H., Trinh, T. Q., Groenen, M., Komen, H., Megens, H.J.W.C 2020QTLs controlling swimming performance and their effect on growth in Nile tilapia (*Oreochromis niloticus*). WIAS Annual Conference 2022

Yu, X., Megens, H.J.W.C., **Mengistu, S.**, Groenen, M. 2020. Genome-Wide Association Analysis of adaptation to oxygen variations in Nile Tilapia (*Oreochromis niloticus*). WIAS Science Day. Lunteren, the Netherlands.

**Mengistu, S. B.**, Mulder, H. A., Benzie, J. A. H., Khaw, H. L., Komen, H. 2019. Non-aerated ponds reduces variances and heritabilities compared to aerated ponds in Nile tilapia. 70$^{th}$ Annual Meeting of the European Federation of Animal Science (EAAP). Ghent, Belgium.

**Mengistu, S. B.**, Mulder, H. A., Benzie, J. A. H., Komen, H. 2019.Genetic parameters for resilience based on fluctuations in body weight in Nile tilapia grown in aerated and non-aerated ponds. European Aquaculture Society (EAS). Berlin, Germany.

**Mengistu, S. B.**, Mulder, H. A., Benzie, J. A. H., Khaw, H. L., Komen, H. 2018. Genotype by environment interaction for growth and survival of Nile tilapia in aerated and non-aerated ponds. International Symposium of Genetics in Aquaculture (ISGA). Cairns, Australia.

**Mengistu, S. B.**, Mulder, H. A., Benzie, J. A. H., Komen, H. 2017. A systematic review of management factors affecting growth, feed conversion ratio and survival in Nile tilapia. European Aquaculture Society (EAS). Dubrovnik, Croatia.

## Training and Supervision Plan (TSP)

| The Basic Package (3 ECTS) | year |
|---|---|
| WIAS Introduction Day | 2016 |
| Ethics and Philosophy in Animal Sciences (RI&EA) | 2016 |
| Course on essential skills | 2018 |

| Disciplinary Competences (20.4 ECTS) | year |
|---|---|
| Design and implementation of breeding programs for smallholder farmers, ILRI, Addis Ababa, Ethiopia | 2015 |
| Modern statistics for life science | 2016 |
| Design of Experiments | 2016 |
| Mixed Linear Models | 2016 |
| Meta-analysis | 2016 |
| Introduction to R for Statistical Analysis | 2016 |
| Literature review | 2017 |
| Linear Models in Animal Breeding, NOVA course, Sweden | 2018 |
| Quantitative Genetics Discussion Group (QDG) | 2016 & 2018 |
| Getting started in ASReml | 2019 |

| Professional Competences (4.8 ECTS) | year |
|---|---|
| The Essentials of Scientific Writing and Presenting (ESWP) | 2016 |
| Project and Time Management (PTM) | 2017 |
| Information Literacy including EndNote Introduction (ILP) | 2017 |
| Critical thinking and argumentation | 2018 |
| Supervising BSc & MSc thesis students | 2018 |
| The final touch: Writing the general introduction and discussion | 2019 |

| **Presentation Skills *(maximum 4 credits)*** | year |
|---|---|
| Systematic review of management factors affecting growth, feed efficiency and survival in Nile tilapia. European Aquaculture Society (EAS),Dubrovnik, Croatia, Oct. 17-20,2017. (Poster) | 2017 |
| Genotype by environment interaction for growth and survival of Nile tilapia in aerated and non-aerated ponds. International Symposium on Genetics in Aquaculture (ISGA), Cairns, Australia, July 15-20, 2018. (Poster) | 2018 |
| A systematic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia (*Oreochromis niloticus*). Sustainable Aquaculture Flagship Science Hour, Penang, Malaysia, July 12, 2019. (Oral) | 2019 |
| Genetic parameters for resilience based on fluctuations in body weight in Nile tilapia grown in aerated and non-aerated ponds. European Aquaculture Society (EAS), Berlin, Germany, Oct. 7-9, 2019. (Oral) | 2019 |
| Non-aerated ponds reduces variances and heritabilities compared to aerated ponds in Nile tilapia. European Association of Animal Production (EAAP), August 26 - 30, 2019, Ghent, Belgium. (Poster) | 2019 |

| **Teaching competences *(max 6 credits)*** | **year** |
|---|---|
| Assisting Genetic Improvement of Livestock (ABG31306) course | 2019 |

| **Education and Training Total (minimum 30 credits)*** | **34.2** |
|---|---|

*One ECTS credit equals a study load of approximately 28 hours

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