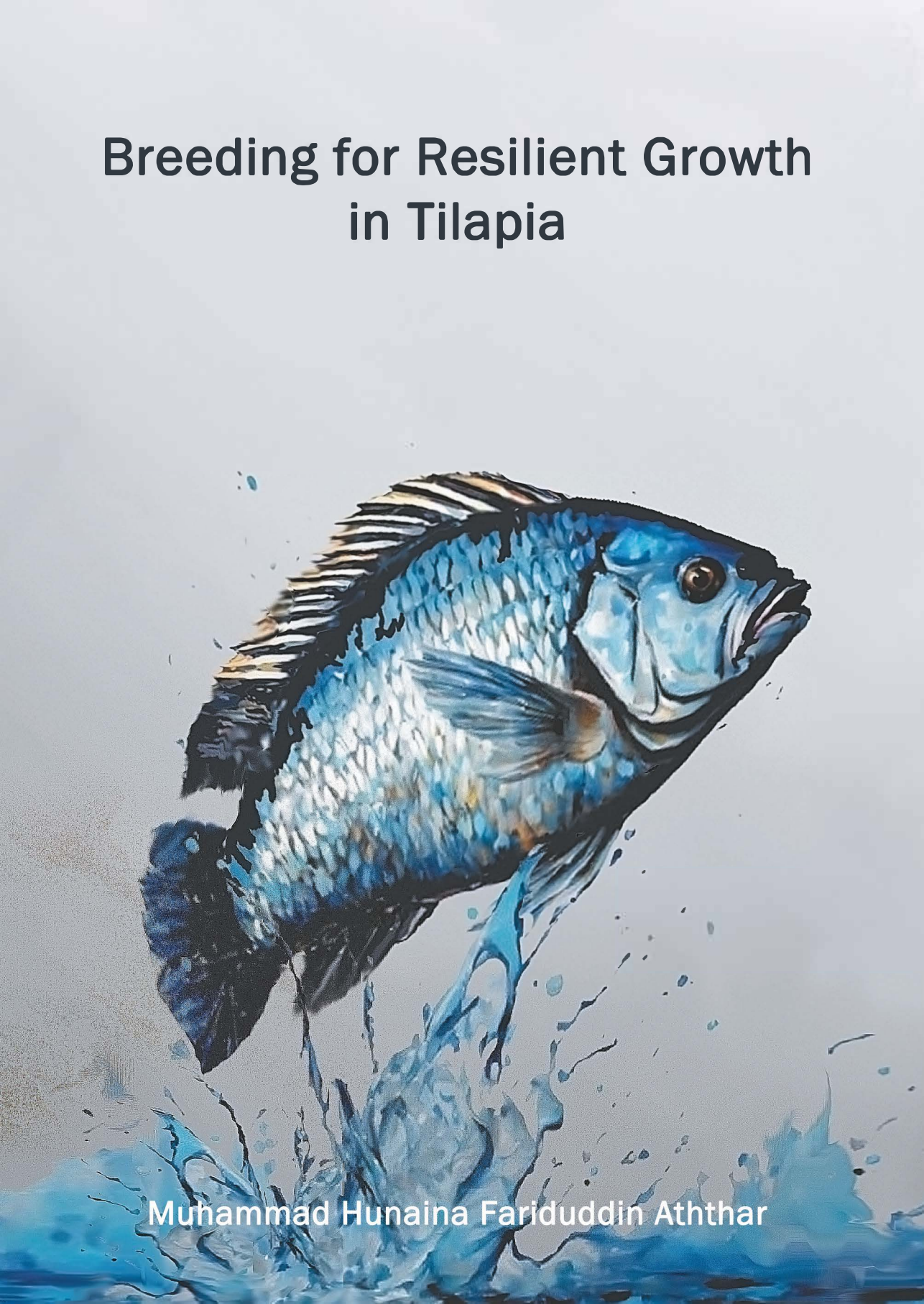
Breeding for Resilient Growth in Tilapia



Muhammad Hunaina Fariduddin Aththar

Propositions

1. Variations during growth of individual fish, are a sensitive measurement to test genotype by environment interaction (GxE) for growth.
   (this thesis)

2. Aquaculture species grown in polyculture systems have higher variation in individual growth.
   (this thesis)

3. It is necessary to frame the debate on animal welfare from a scientific perspective rather than from a public perspective.

4. Eating less meat in developing countries will have very little effect on global greenhouse gas emissions.

5. Shortening the working week is a more effective and efficient way to increase productivity than shifting to remote working.

6. The use of Video Assisted Referee (VAR) makes football boring.

7. If artificial intelligence (AI) can replace the specialist, then the specialist should specialize even more.

Propositions belonging to the thesis, entitled
Breeding for resilient growth in tilapia

Muhammad Hunaina Fariduddin Aththar
Wageningen, 2 December 2024

Breeding for resilient growth in tilapia

Muhammad Hunaina Fariduddin Aththar

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

Breeding for resilient growth in tilapia

Muhammad Hunaina Fariduddin Aththar

Thesis

Submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr C. Kroeze,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 2 December 2024
at 3.30 p.m. in the Omnia Auditorium.

Muhammad Hunaina Fariduddin Aththar
Breeding for resilient growth in tilapia,
160 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2024)
With references, with summary in English

ISBN 978-94-6510-329-7
DOI https://doi.org/10.18174/676309

ABSTRACT

Aththar, MHF. (2024). Breeding for resilient growth in tilapia. PhD thesis, Wageningen University & Research, the Netherlands.

The dynamic environments and wide diversity of aquaculture production systems along with an increase from less to more intensive production systems can introduce various biotic and abiotic stressors. It is important for farmers to have fish that show more consistency in growth despite environmental stressors. Longitudinal weight records can be used to measure growth consistency which is an indicator for the ability of fish to recover to baseline levels following stressors. The aim of this thesis is to improve methods for measuring resilient growth using the variance of deviations from the expected growth performance (*LnVar*) and to provide knowledge for improving resilient growth in tilapia through selective breeding. First, I addressed the development of a novel measure for *LnVar* using individual growth curves as the expected performance ($LnVar_{ind}$) and used this to estimate the genetic correlation between *LnVar* and growth in freshwater ponds with or without daily diurnal dissolved oxygen fluctuations. $LnVar_{ind}$ was found to be highly heritable in the more challenging environment and this can be exploited by selective breeding. The genetic correlation of $LnVar_{ind}$ between the ponds was 0.50, suggesting that genetic improvement in the good environment will not automatically lead to improved $LnVar_{ind}$ in the challenging environment. Second, I defined the economic value of *LnVar* based on the effect of fluctuation in fish growth on feed waste, growth deficiency and feed saving. This shows that reducing *LnVar* will improve economic returns. Incorporating *LnVar* into the selection index alongside *HW* increased the economic response by 11%. Third, I addressed the genotype by environment interaction for growth of Sukamandi tilapia between freshwater and brackish water ponds. Brackish water resulted in higher growth performance and there was substantial GxE interaction for growth between brackish water and freshwater. Fourth, I estimated genetic parameters of growth consistency measured with *LnVar*, in brackish water monoculture and in co-culture with shrimps. Sukamandi tilapia is able to thrive in co-culture with shrimps, achieving growth rates comparable to monoculture. We found heritable variation in *LnVar* for tilapia grown in the brackish water ponds. We found moderate GxE between co-culture and monoculture. The magnitude of GxE for *LnVar* between co-culture and monoculture is higher than that for the growth parameters, suggesting that *LnVar* is more responsive to the environmental differences than growth. The genetic correlation between *LnVar* and the growth of Sukamandi tilapia is less than unity, which supports the idea that *LnVar* and growth are different traits. Finally, I present a broader discussion on the concept of growth consistency as an indicator for resilience and its application in fish breeding programs.

Contents

Chapter 1

General Introduction

1.1 Fish growth and resilience

Growth is considered the paramount economic trait in aquaculture and a top priority for farmers. Accelerated growth has the potential to shorten culture cycles, leading to increased profits (Janssen et al., 2017a). Harvest weight and growth rate are the key indicators for evaluating growth performance. Harvest weight indicates the target market size and growth rate provides information on the length of the culture cycle until fish reach market size (Hopkins, 1992; De Graaf and Prein, 2005; Lugert et al., 2016). Growth is one of the most important selection traits and is a highly heritable trait in many fish species, with selection for a faster growth rate allowing for increased production (Dunham, 2011; Chavanne et al., 2016; Gjedrem and Rye, 2018; Houston et al., 2022).

In aquaculture production, water quality heavily influences fish growth due to their reliance on the ambient environment (Boyd, 2017). Changes in fish growth performance can result from physiological adaptation to environmental stressors (Barton, B. A., 2002; Bœuf and Payan, 2001). Exposure to a concentration of one or more water-quality variables (oxygen, temperature, salinity) that is higher or lower than optimal can lead to stress, which may partition the energy substrate away from growth (Barton, Bruce A., 2002; Guderley and Pörtner, 2010), leading to decreased fish production (Wedemeyer, 1996; Boyd, 2017). Stress may modify the energy fluxes from the nutrients entering the fish via various mechanisms, such as reduced feed intake, limited food absorption in the gut, and increased energy allocation for maintenance processes (Sadoul and Vijayan, 2016), with limited available energy for growth at a given moment. For example, Wang et al. (1997) showed that as salinity increased, fish daily weight gain decreased and the rate of ammonia excretion increased. This suggests that fish diverted energy away from growth and towards the maintenance of homeostasis.

This theory on the effect of stressors on growth also predicts that animals less affected by environmental stressors can show more consistency in production performance, as has been shown for chicken, pigs and dairy cows (Colditz and Hine, 2016; Berghof et al., 2019a; Mengistu et al., 2021; Poppe et al., 2020). This capacity to cope with stressors and maintain performance even amidst environmental stressors is also known as animal resilience (Colditz and Hine, 2016). Resilience of fish can differ based on the nature of the environmental stressors, e.g., heat stress and low oxygen stress. Thus, the definition of resilience is generally seen as a composite trait consisting of different resilience types, such as heat stress-resilience and hypoxia stress-resilience (Colditz and Hine, 2016; Friggens et al., 2017 et al., 2017; Berghof et al., 2019b; Mengistu et al., 2022).

In the context of aquaculture, where growth is the primary production trait of interest, it is important to select fish that consistently grow well despite environmental stressors that are typical for the dynamic and diverse environments in aquaculture production systems or increasingly severe weather extremes caused by climate change (Reid et al., 2019; Dabbadie et al., 2019b; Soto et al., 2018; Sae-Lim et al., 2017; Agha et al., 2018).

1.2 Genetic selection to improve fish resilience

Resilience, measured as the consistency in growth, can be calculated from the deviation of actual weight from the expected weight in longitudinal measurements (Mengistu et al., 2022; Berghof et al., 2019a). Several indicators to measure resilience from the deviation of actual weight have been proposed, including natural logarithmic of variance of deviations between observed and expected performance (*LnVar*), autocorrelations between measurements, skewness of deviations or a slope of reaction norm (Berghof et al., 2019b). Of these, *LnVar* is the most promising indicator to measure resilience trait based on its moderate heritability and ease of calculation from longitudinal records (Elgersma et al., 2018; Berghof et al., 2019b; Mengistu et al., 2022; Gorssen et al., 2023). In fish, *LnVar* can be calculated from the deviations between observed and expected performance from longitudinal records on body weight. Ideally, an expected growth curve would be as close as possible to the curve that an animal would have realized without disturbances. More resilient animals are expected to show lower *LnVar* values than less resilient animals.

Growth consistency, measured as *LnVar*, has moderate heritability, indicating the presence of sufficient additive genetic variance for future selection. A previous study by Berghof et al. (2019a) and Mengistu et al. (2022), calculated *LnVar* from the expected cohort growth in chickens and tilapia and found a heritability of 0.10. However, how animals respond to environmental stressors can differ at the individual level (Schreck, 2000; Bœuf and Payan, 2001; Kültz, 2015) and individual deviations are important when studying responses to the environmental stressors (Wendelaar Bonga, 1997; Van Weerd and Komen, 1998; Silva et al., 2010). It is crucial to estimate a reference or expected performance that remains independent of environmental conditions. This would ensure that deviations from the expected performance provide the most valuable insights into how the individual responds to stressors. Consequently, these deviations could be used as indicators of resilience. The change in mean cohort weight still depends on the environmental conditions between time points. Consequently, estimating the response of each fish based on deviation from the mean weight of the fish cohort could mask the impact of environmental changes on fish at the individual level. An alternative approach involves fitting an expected individual growth curve

from longitudinal measurements of fish weights that is expected to be less dependent from environmental conditions. Here, the hypothesis is that deviations from an individual's expected growth can accurately capture the response of individual fish to environmental stressors and contribute to a higher heritability of *LnVar*.

Selection for increased growth may result in undesired correlated responses, such as animals with a high growth rate becoming more sensitive to environmental stressors. Given the dynamic and diverse environments in aquaculture production systems, including resilience in the breeding goal is potentially beneficial to a successful breeding program. Mengistu et al. (2022) showed an unfavourable correlation between *LnVar* measured from expected cohort weight and growth in tilapia that were grown in ponds without aeration. A balanced breeding goal with resilience and growth is then needed. A simulation from Berghof et al. (2019b) showed that including a resilience indicator in the selection index of pigs can result in a higher selection response in the breeding goal and more resilient animals. However, more studies investigating this relationship are needed.

1.3 Genotype-by-environment interaction (GxE) and fish resilience

Fish breeding strives to create populations that perform well under various aquaculture commercial production circumstances. Fish breeding and reproduction structures typically consist of a breeding nucleus where genetic gain is generated, as well as a multiplier and the grow-out units. The breeding candidates are usually reared at a single breeding nucleus farm and may be kept in the aquaculture facility under relatively controlled environmental conditions. However, the environmental conditions in the nucleus are often different from the grow-out conditions for the commercial markets that may extend across multiple production environments and systems. Production systems are be highly diverse ranging from extensive to super-intensive, from freshwater to brackish water, and from net-pen and floating cages to ponds and closed system tanks (RAS) (Verdegem et al., 2023; Naylor et al., 2021). These different environments can present significantly different physiological challenges to the fish. In addition, the continuum of increasing densities from less intensive production systems to more intensive production systems affects water quality and can lead to stress and dysfunction in the fish if they are at levels approaching or beyond the average tolerance capacity of fish (Tidwell, 2012; Schreck and Tort, 2016). The most common water quality variables causing stress in aquaculture animals are temperature, salinity, and pH, which may be either too low or too high; low DO concentration; high concentrations of carbon dioxide; and toxic concentrations of ammonia, nitrogen and nitrite (Boyd, 2017).

Genotype-by-environment interaction (GxE) occurs when different genotypes respond differently to these variations in environmental conditions. Significant GxE for growth has been reported not only across different aquaculture production systems but also within the same production system under diverse environmental conditions and across different geographical locations due to the global distribution of genetically improved fish (Table 1.1).

LnVar measures the growth consistency in challenging environments. We hypothesize that *LnVar* shows higher heritability in more challenging environments, suggesting that it is genetically more expressed in stressful conditions. In other words, the challenging environment may amplify the expression of *LnVar*'s genetic potential compared to non-challenging environments. The differential expression or variation in animal responses to environmental conditions will lead to the reordering of individual performance rankings between stressful and stable environments. We hypothesize that in the presence of GxE for growth, *LnVar* will also be affected. Alternatively, *LnVar* could act as an indicator of GxE interaction for growth between contrasting environments.

Table 1.1 Genotype by production environment (GxE) interaction studies for growth of aquaculture species

Species	Production system, environments or locations	Genetic correlation (r_g)	Reference
Penaeus monodon	Indoor recirculating systems and outdoor ponds	0.17 - 0.31	Sang et al., 2020
Sole	Intensive recirculation system and semi-natural outdoor pond	0.56 ± 0.34	Mas-Muñoz et al., 2013
Nile tilapia - GIFT	Pond and cage	0.36 - 0.82	Eknath et al., 2007
Nile tilapia - GIFT	Pond and cage	0.70	Ponzoni et al., 2011
Nile tilapia - GIFT	Pond and cage	0.73	Khaw et al., 2012
Red tilapia	Pond and cage	0.85 - 0.90	Nguyen et al., 2017
Nile tilapia - GIFT	Pond and cage	0.47	de Araújo et al., 2020
Nile tilapia	Bio floc, recirculating water and cage systems	0.59 - 0.88	Turra et al., 2016
Nile tilapia - GIFT	Low and high input pond	0.74 - 0.84	Khaw et al., 2009
Nile tilapia	Cage with commercial pellet feed and rice-fish culture	0.53 - 0.98	Bentsen et al., 2012

Nile tilapia - GIFT	Pond, cage and VAC (garden, pond and livestock pen)	0.86 - 0.94	Trọng, T.Q. et al., 2013
Nile tilapia	Bio floc, recirculation and cage systems	0.6 - 0.7	Fernandes et al., 2019
Nile tilapia	Low input and high input pond	0.95	Workagegn et al., 2020
Seabass	Recirculating system, a concrete raceway with well water, semi-intensive estuarine earthen ponds and tropical seawater cages	0.21 - 0.61	Dupont-Nivet et al., 2010
Rainbow trout	Nucleus, freshwater recirculating aquaculture system; a high-altitude farm and a cold-water farm	0.15 - 0.37	Sae-lim 2013
Nile tilapia - GIFT	Aerated and non-aerated pond	0.78 - 0.81	Mengistu et al., 2020a
Atlantic salmon	Seawater and freshwater	0.26 - 0.31	Gonzales et al, 2022
Barramundi	Marine and freshwater	0.81 ± 0.11	Domingos et al 2021
Nile tilapia	Freshwater and brackish water	0.92	Thoa et al., 2016
Nile tilapia - GIFT	Brackish and freshwater pond	0.45	Luan et al., 2008a
Nile tilapia	Tank with temperature 15–20°C and 20–25°C	0.84	Thoa et al., 2016
Nile tilapia - GIFT	Between countries (China, Malaysia and India)	0.33 - 0.71	Agha et al., 2018
Seabream	Sea cage in Greece and Spain	0.43 - 0.45	Gulzari et al., 2022

1.4 The investigation of resilience in tilapia

Fast growth, resistance to stress and disease, and tolerance to a wide range of temperatures, DO levels, and salinity levels are the key attributes that make Nile tilapia an outstanding aquaculture species (El-Sayed, 2020; El-Sayed and Fitzsimmons, 2023). Global Nile tilapia production increased by 4.4 times from 2000 to 2020, contributing 68% to total Asian tilapia production and 45% to global tilapia output (FAO, 2022). Many selective breeding programs for Nile tilapia have been established up to now (Pullin, 1988; Eknath et al., 1993; Bentsen et al., 1998; Bolivar, 1998; Zimmermann and Natividad, 2004; Tayamen, 2004; Thodesen et al., 2011; Neira, 2010; Ponzoni et al., 2011) but few breeding programs have investigated ways to improve productivity in low input farms or farms with severe environmental stressors (Luan et al., 2008a; Trọng, T.Q. et al., 2013; Workagegn et al., 2020; Mengistu et al., 2020a).

The GIFT strain breeding program, led by WorldFish Malaysia, and the Sukamandi strain breeding program, conducted by Research Institute for Fish Breeding (RIFB) Indonesia, focus on improving tilapia productivity in challenging environments. The GIFT strain was selected under optimal DO conditions, while smallholder production occurs in non-aerated earthen ponds. During the grow-out period, DO level consistently exceeds 5 mg/l in aerated ponds, whereas in non-aerated ponds, DO level may drop to <1 mg/l at night (Mengistu et al., 2020a). Prolonged exposure to hypoxia (DO < 3 mg/l) is known to suppress fish growth due to reduced feed intake, limited metabolic processes (Magnoni et al., 2018; Brauner and Richards, 2020) and may result in increased susceptibility to disease (Douxfils et al., 2014; Abdel-Tawwab et al., 2019; Wang et al., 2023). In Indonesia, RIFB has been conducting a small-scale breeding program for salinity tolerance using the Sukamandi tilapia, a unique tilapia strain composed of Nile tilapia (*Oreochromis niloticus*) and blue tilapia (*Oreochromis aureus*), to optimize growth in brackish water (Yu et al., 2022). Candidate parents for the next generation are selected in freshwater ponds based from their sibs information in brackish water ponds, while production takes place in brackish water ponds with daily salinity fluctuating between 6-25 ppt. The salinity fluctuation in the brackish water area of north Java coastal regions in Indonesia is mainly caused by variations in daily rainfall volume (Ariadi et al., 2023; Mahasin et al., 2020; As-syakur et al., 2013). Salinity level fluctuations pose a challenge to aquaculture productivity in brackish water areas. Fish require energy to maintain osmotic homeostasis in the environment with salinity fluctuations (Kültz, 2015; Bal et al., 2021). However, with limited available energy, the response of fish to environmental changes potentially diverts energy substrates away from growth, thereby reducing fish production performance. Both the GIFT and Sukamandi strain breeding programs record tilapia growth performance in the challenging environments characterized by daily diurnal oxygen and salinity fluctuation, respectively. In this thesis, we used growth data from GIFT tilapia grown in non-aerated ponds and Sukamandi strain tilapia grown in brackish water ponds to investigate *LnVar* as a resilience indicator and to estimate genetic parameters of *LnVar* in Nile tilapia.

1.5 Aim and outline

Growth consistency can be measured by *LnVar*. *LnVar* has been calculated from deviations from the mean growth of the animal cohort (Berghof et al., 2019a, Mengistu et al., 2022). However, deviations from expected individual growth are assumed to more accurately capture the response in the performance of individual fish to environmental stressors than the mean growth of the cohort. Estimating the heritability of *LnVar* based on expected individual performance and its genetic correlation with growth as the primary production

trait is important for improving resilience through selective breeding. Additionally, *LnVar* should be more highly expressed in stressful environments and contribute to substantial GxE interaction for growth between stable and stressed environments.

Breeding programs for salinity-tolerant tilapia in Indonesia have relied on mass selection in brackish water ponds, exposing selection candidates to risks such as poor biosecurity, high mortality and expensive transportation from production locations to the nucleus hatchery. An alternative approach involves a nucleus-based breeding program, selecting candidate parents in freshwater ponds. However, significant differences in salinity levels between the nucleus and production environment may occur when production is conducted in brackish water ponds, potentially leading to differences in tilapia productivity between freshwater and brackish water ponds. Such mismatches between genotype and environment can result in GxE interactions (Falconer and Mackay, 1996). If there is significant GxE interaction, the performance of candidates in nucleus may not predict their performance in test environments or production system. In addition to a nucleus, a breeding program can also include separate test environments located in alternative production systems. Furthermore, an impact of salinity on the genetic parameters of Nile tilapia growth is expected.

I applied resilience based on the expected individual performance of Nile tilapia grown in brackish water environments with daily salinity fluctuations. Knowledge of heritability and genetic correlations with growth is essential for improving the resilience of Nile tilapia in brackish water environments and the simultaneous improvement of resilience and growth without unintended trade-offs through selective breeding.

Finally, we hypothesize that economic advantages are expected from selection on *LnVar* through the indirect benefits of resilience on improved feed efficiency. In this thesis, we explore ways to calculate economic values of resilience indicators based on reduced feed cost. Fish that consistently perform well are crucial for enhancing feed efficiency in aquaculture. Farmers determine feeding requirements based on growth prediction (Dumas et al., 2007). When fish exhibit more consistent growth, feeding requirements can be estimated more precisely, resulting in increased feed efficiency and economic advantages.

The aim of this thesis is to improve methods for measuring resilient growth and to provide knowledge for improving the resilient growth in tilapia through breeding program. The specific objectives of this thesis are:

1. To develop a novel measure of *LnVar* using individual growth curves as expected performance
2. To estimate the genetic correlation between *LnVar* and growth in freshwater ponds with or without daily diurnal dissolved oxygen fluctuations.
3. To investigate the economic value of *LnVar* and the potential of economic gain from *LnVar* using selective breeding.
4. To estimate the genotype-by-environment interaction for growth between freshwater ponds and brackish water ponds and the impact of the presence of shrimp on these genetic parameters.
5. To estimate the genetic parameters for *LnVar* and the correlation between *LnVar* and growth in brackish water ponds with strong salinity and temperature fluctuations.

The structure of the research chapters in this thesis is summarized in Figure 1.1. In **Chapter 2**, I present an improved method, which estimates daily growth coefficient by regressing five weight records on age and an improved definition of *LnVar* from expected individual weight to better capture the response in performance of individual fish to the environmental stressors. We estimate the heritability of *LnVar* based on the expected individual weight and its genetic correlation with growth in freshwater ponds that experience daily diurnal DO fluctuation. **Chapter 3** focuses on deriving economic values for *LnVar* and exploring the potential of economic gain from *LnVar* using selective breeding. In **Chapter 4,** we investigate the presence of GxE between freshwater and brackish water ponds and the impact of salinity on genetic parameters for growth of tilapia. In **Chapter 5**, we apply the improved method of calculating *LnVar* based on the individual expected growth trajectories and estimate genetic parameters for growth and *LnVar* in brackish water co-culture and monoculture. We report genetic correlations of growth and *LnVar* between co-culture and monoculture treatments and correlation of *LnVar* with growth. **Chapter 6**, the final chapter of this thesis, consists of four sections. The first section describes the source of stressors in aquaculture. The second section discusses the growth consistency as a resilience indicator. In the third section, I explore the application of *LnVar* in fish breeding programs. The final section discusses whether breeding should focus on fish with low or high *LnVar*.

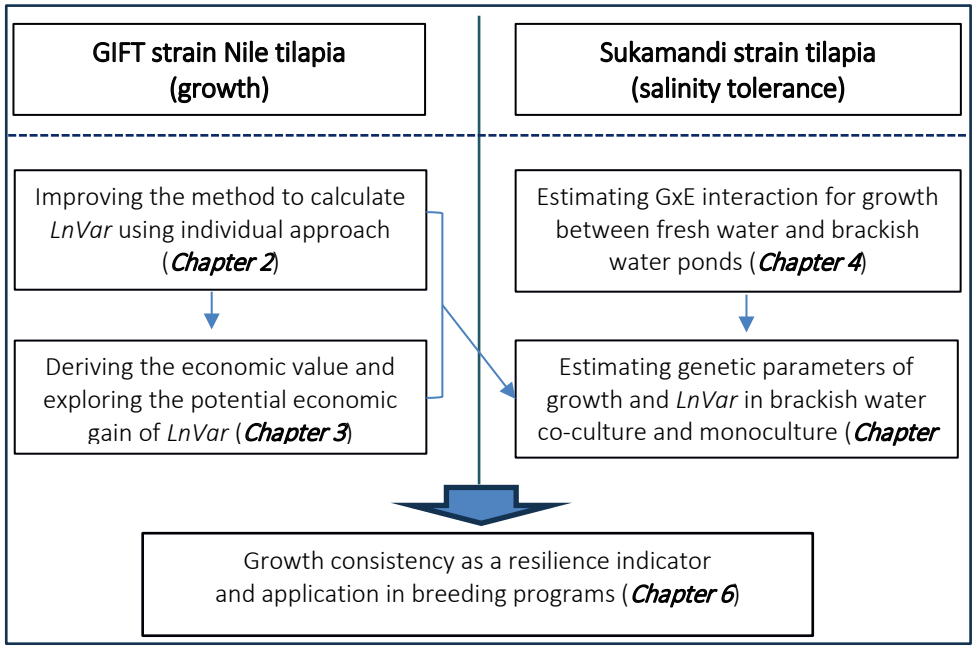

Figure 1.1. Structure of the research chapters in this thesis.

Chapter 2

Log transformed variance (*LnVar*) from individual growth curves as a potential indicator of resilience in Nile tilapia (*Oreochromis niloticus*)

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Under review in Scientific Reports

Abstract

The ability of the animal to cope with environmental changes may be measured by log-transformed variance of deviations from expected weights (*LnVar*). We calculate *LnVar* by fitting the expected individual growth curve based on longitudinal weights ($LnVar_{ind}$) of Nile tilapia that were grown in either an aerated or a non-aerated freshwater pond. We estimated genetic parameters for $LnVar_{ind}$ in Nile tilapia, the genetic correlation between $LnVar_{ind}$ and growth and the genetic correlation for $LnVar_{ind}$ between aerated and non-aerated ponds. The heritability estimate for $LnVar_{ind}$ (0.28) in the non-aerated ponds was higher than in aerated ponds (0.06). In the aerated ponds, genetic correlations of $LnVar_{ind}$ were -0.44 ± 0.23 with daily growth coefficient (*DGC*) and -0.45 ± 0.24 with harvest weight (W_5). In the non-aerated ponds, genetic correlations with *DGC* and W_5 were -0.68 ± 0.12 and -0.52 ± 0.17, respectively. These values suggest that selection for fish with high growth rate will also improve $LnVar_{ind}$. However, genetic correlation of $LnVar_{ind}$ between aerated and non-aerated ponds was 0.50, suggesting that genetic improvement in the aerated environment will not automatically improve $LnVar_{ind}$ in the non-aerated environment. Therefore, incorporating records from relatives in non-aerated ponds is beneficial for breeding programs targeting this environment.

Keywords: Variance of deviation of individual growth, Resilience, Nile tilapia, Heritability, Genetic improvement

2.1 Introduction

All animals, including fish, respond to environment stressors with changes in behaviour and increased levels of stress hormones, such as cortisol and adrenalin (Schreck, 2000; Bœuf and Payan, 2001; Kültz, 2015). Stress, either acute or chronic, affects feed intake, digestion, and ultimately growth (Wendelaar Bonga, 1997; Van Weerd and Komen, 1998; Silva et al., 2010). However, the magnitude of these responses can vary significantly between individuals. This has led to the idea that individual deviations in growth over time could be used as an indicator for sensitivity to stressful conditions. Resilience is then defined as the ability of the animal to cope with environmental stressors or to rapidly return to the condition it had before exposure to a stressors (Colditz and Hine, 2016; Mulder and Rashidi, 2017; Scheffer et al., 2018; Berghof et al., 2019b). Resilience is also seen as the ability to have consistent performance throughout time. Animals that show consistent performance are expected to be less affected by environmental stressors than animals with less consistent production. Therefore, resilience indicators can be based on observed production variations even though the causes of these variations are unknown (Scheffer et al., 2018; Berghof et al., 2019b). Resilience, expressed as the consistency in growth, can be measured from the deviations of actual weight from the expected weight in longitudinal measurements (Colditz and Hine, 2016; Friggens et al., 2017). Several indicators to measure resilience from the deviation of actual weight have been proposed (Berghof et al., 2019b). Of these, *LnVar* is the most promising based on its moderate heritability and ease of calculation from longitudinal records (Elgersma et al., 2018; Berghof et al., 2019a; Mengistu et al., 2022).

Ideally, the reference used to calculate individual deviations would be as close as possible to the trajectory that a fish would have realized in the absence of stressors. Therefore, it is important to estimate a reference or expected performance that is independent of environmental conditions. A previous study by Mengistu et al. (2022) measured fish response to the stressors using *LnVar*, calculated based on individual deviations from the mean weight of the fish cohort ($LnVar_{coh}$). However, the changes in the mean weight of the fish cohort are also dependent on environmental conditions across various time points. Consequently, the response of individual fish to the stressors, calculated based on the mean weight of the fish cohort, is relative to the response of the group or cohort to environmental changes. An alternative approach is to fit an expected growth curve from longitudinal measurement of fish weights. Growth curves can be fitted using weight and age in calendar days or

temperature days (Lugert et al., 2016). Feeding levels also affect growth rate but animals in commercial production are typically fed to satiation.

In aquaculture and fisheries, nonlinear functions to model the age–weight relation have been intensively used to describe the growth curve of different aquatic species, including the Gompertz function, von Bertalanffy growth function and Schnute function (Lugert et al., 2016). The application of nonlinear models for fitting growth curves was also helpful in describing the growth in Nile tilapia (Oliveira Zardin et al., 2019). Most of these growth models are based on the metabolic growth model, assuming that growth depends on weight exponent of 2/3 (von Bertalanffy, 1938; Taylor, 1962). von Bertalanffy reasoned that the area of surfaces involved in anabolism is proportional to a linear dimension squared and that the weight related to catabolism is proportional to a linear dimension cubed (von Bertalanffy, 1938; Taylor, 1962). The weight exponent of fish can be estimated from weight data using nonlinear regression. Mayer et al. (2012) and Janssen et al. (2017a) estimated a weight exponent of ~2/3 in Seabream.

In this study, we apply non-linear regression to fit individual growth curves using longitudinal weight measurements of tilapia grown in Malaysia (described in detail in Mengistu et al. (2022)). Tilapia were grown in an aerated and non-aerated freshwater pond and weight was measured at 5-time points during the grow-out period. The individual growth curves were then used to calculate *LnVar* ($LnVar_{ind}$). We hypothesize that *LnVar* measures growth resilience and therefore should be expressed more in the non-aerated environment due to high fluctuations in dissolved oxygen levels. The objectives of this study were: 1) to estimate genetic parameters of $LnVar_{ind}$ in Nile tilapia (*Oreochromis niloticus*), 2) to estimate the genetic correlation between $LnVar_{ind}$ and growth to explore the effects of selection for growth rate on $LnVar_{ind}$ and 3) to estimate the genetic correlation for $LnVar_{ind}$ between aerated and non-aerated ponds.

2.2 Materials and Methods

The experiment was conducted in the Aquaculture Extension Centre, Department of Fisheries, Jitra, Kedah State, Malaysia. The source of the experimental fish is the Genetically Improved Farmed Tilapia (GIFT) Breeding Program that is run by WorldFish in Malaysia. The details of family production and grow-out of tilapia were described by Mengistu et al.

(2020a). Below, we summarize family production, nursery, and grow-out of tilapia for this study.

2.2.1 Family production

We produced our experimental fish using the 16th generation of the GIFT strain as selected parents. We maintained the male and female breeders in separate 9 m2 hapas (3 m × 3 m) with a mesh size of 1cm in an earthen pond for two weeks. Mating was done in four hapas (each 30m2) suspended in a 500m2 earthen pond. Eighteen males and 50 female breeders were stocked in each of the mating cages. In total, 72 males and 200 females were used. We conducted this mating process for 15 days. On the sixteenth day, the parents were removed, and the fry were kept in the same cages for a nursing period of 60 days.

2.2.2 Grow-out period

The fingerlings from each net cage were transferred into one of four aerated tanks after the 60 days nursing period and conditioned for three days before tagging. A random sample of fingerlings was anesthetized using clove oil and individually tagged using PIT (passive integrated transponder) tags. At tagging, a 1 cm2 fin clip sample was collected and PIT tag number and body weight were recorded. Equal numbers of individually tagged fingerlings from each nursery cage were randomly allocated to two earthen ponds. In total, 1570 fish were stocked in each pond with a stocking density of 3 fish/m2. The size of each of the ponds was 511m2 with a water depth of 1 to 1.2 meters. To test the effect of oxygen availability on resilience in growth, we created two different environments: One of the ponds was aerated using a paddle wheel and blower to create a normoxic environment. The second pond was without aerators which resulted in natural diurnal dissolved oxygen (DO) fluctuations.

2.2.3 Trait measurements

Longitudinal measurement

We measured the weight of tilapia at five time-points: at stocking (W_1: day 1), three interval time points ($W_{2\text{-}4}$): 55, 104 and 167 days) and at harvest (W_5: 217 days). Fish from the non-aerated and the aerated pond were always measured on two consecutive days.

The calculation of *LnVar* needs the expected performance from which the observed deviation can be calculated. In this study we calculated *LnVar* from the individual expected growth

trajectories ($LnVar_{ind}$). We compared $LnVar_{ind}$ to the calculation of *LnVar* as used by Mengistu et al. (2022) (hereafter cohort approach: $LnVar_{coh}$). The methods differ in their approach to calculate the expected performance.

Calculation of $LnVar_{ind}$

$LnVar_{ind}$ was calculated from the deviations of observed weights from the expected weights of the individual at timepoints W_1 to W_5. To obtain the expected weights we fitted an exponential curve to the observed weights:

$$W_{it} = a + b_i * t^f \quad \text{Eq. [2.1]}$$

where W_{it} is the weight of fish *i* at age *t*, a is the intercept, b_i is the slope of the non-linear regression for fish *i*, *t* is the fish age and *f* is the overall weight exponent. The growth curve exponent *f* was estimated for the fish in this experiment using the nls function in R (RStudio-Team, 2022). The non-linear regression coefficient (b_i) obtained from Eq. [2.1], is equivalent to the daily growth coefficient (*DGC*) per fish. Then, we transformed the five observed weights per fish as $W^{\frac{1}{f}}$ to linearize the growth curve and we estimated *DGC* per fish as the slope of the linear regression of $W^{\frac{1}{f}}$ on the age of the fish at the five time points t and calculated the expected weight of individual fish at times *t*:

$$W_{exp,it}^{\frac{1}{f}} = a + DGC_i * t \quad \text{Eq. [2.2]}$$

where $W_{exp,\,it}$ is the expected weight of fish *i* at age *t*, a is the intercept, DGC_i is the daily growth coefficient as the slope of the non-linear regression for fish *i*, *t* is the fish age. Per fish, we then calculate *LnVar* from the deviations (dev_{it}) as:

$$dev_{it} = W_{obs\ it}^{\frac{1}{f}} - W_{exp\ it}^{\frac{1}{f}} \quad \text{Eq. [2.3]}$$

Where dev_{it} is the deviation of observed weight from expected weight of fish i at time point t, $W_{obs\ it}$ is the observed body weight of fish *i* at time point *t* and $W_{exp\ it}$ is the expected body weight of fish i at time point *t*. Next, for each fish, we calculated variance of the resulting five deviations (Var-dev) and transformed the Var-dev using the natural logarithm to obtain $LnVar_{ind}$.

To investigate the effect of the heteroscedasticity for the deviations between time points, we compared the deviations that were calculated at *1/f* scale in Eq. [2.3] with the deviations that were calculated at *f* scale (observed) and at *1/3* scale (Eq. [2.4] and [2.5], respectively). The growth exponent 3 is commonly used for fitting growth curves of fish with rounded shapes that grow in volume (Bureau, D.P. et al., 2000; Iwama and Tautz, 1981; Jobling, 2003; Lugert et al., 2016). The deviations (dev_t) from *f* scale were calculated as:

$$dev_t = W_{obs\,t} - W_{exp\,t} \quad \text{Eq. [2.4]}$$

Then, the deviations (dev_t) from cubic scale were calculated as:

$$dev_t = W_{obs\,t}^{\frac{1}{3}} - W_{obs\,t}^{\frac{1}{3}} \quad \text{Eq. [2.5]}$$

Where $W_{obs\,t}$ is the observed body weight at *t* and $W_{exp\,t}$ is the expected body weight at *t*.

Calculation of LnVar$_{coh}$

The expected performance for $LnVar_{coh}$ is defined as the mean weight of fish that belong to the same cohort (Mengistu et al., 2022). Fish cohort is defined as the fish belonging to the same nursery hapa, sex and grow-out pond. The details calculation for $LnVar_{coh}$ were described by Mengistu et al. (2022). We refer to *LnVar* based on expected weights from the cohort as $LnVar_{coh}$.

2.2.4 Genetic parameter estimation

Records from 1686 genotyped fish were available for genetic analyses. Genomic relationship matrix was computed based on 11,293 SNPs using the calc_grm program (Calus and Vandenplas, 2016) with the vanraden2 option, as described in Mengistu et al. (2022). Phenotypic and genetic variances of $LnVar_{ind}$, $LnVar_{coh}$, *DGC* and harvest weight (W_5) were estimated using ASReml version 4.2 (Gilmour, A. R. et al., 2015) fitting a bivariate animal model with a genomic relationship matrix. Phenotypic (r_p) and genetic (r_g) correlations between the four traits within the aerated ponds and within the non-aerated ponds were estimated from bivariate linear models. We used the following animal model:

$$y_{ijk} = \mu + \mathrm{CAGE}_i + \mathrm{SEX}_j + \mathrm{SW}_k + \mathrm{a}_k + \mathrm{e}_{ijk} \quad \text{Model [2.1]}$$

where: $\mathbf{y_{ijk}}$ is the vector of $LnVar_{ind}$, $LnVar_{coh}$, *DGC* and W_5 for the univariate models or two of those traits for the bivariate models; μ is overall mean; $\mathbf{CAGE_i}$ is fixed effect that accounts for

nursery hapa effects (i=1– 4); $\mathbf{SEX_j}$ is the fixed effect of sex (j= male, female, unknown); $\mathbf{SW_k}$ is a covariate start-weight of the k-th individual (included only for estimation of harvest weight: W_5); $\mathbf{a_k}$ is random additive genetic effect of the k-th individual; $\mathbf{e_{ijk}}$ is random residual effect associated with an individual. We calculated the heritability as the ratio between additive genetic variance (σ_A^2) and phenotypic variance (σ_P^2), $\frac{\sigma_A^2}{\sigma_P^2}$.

Genetic and residual correlations between traits in the same environment were obtained from bivariate analysis. The animal effects for bivariate model were distributed as N(0,G⊗C) with the additive genetic variance covariance matrix (C) is $\begin{bmatrix} \sigma_{A,1}^2 & r_{A,12}\sigma_{A,1}\sigma_{A,2} \\ r_{A,12}\sigma_{A,1}\sigma_{A,2} & \sigma_{A,2}^2 \end{bmatrix}$, and G is the genomic relationship matrix, $\sigma_{A,1}^2$ and $\sigma_{A,2}^2$ is the additive genetic variance of trait 1 and trait 2. $r_{A,12}\sigma_{A,1}\sigma_{A,2}$ is the additive genetic covariance between trait 1 and trait 2. The residuals were distributed as N(0, I⊗R) with residual variance-covariance matrix (R) is $\begin{bmatrix} \sigma_{e,1}^2 & r_{e,12}\sigma_{e,1}\sigma_{e,2} \\ r_{e,12}\sigma_{e,1}\sigma_{e,2} & \sigma_{e,2}^2 \end{bmatrix}$, where I is an identity matrix, $\sigma_{e,1}^2$ ($\sigma_{e,2}^2$) is the residual variance of trait 1 (trait 2), and $r_{e,12}\sigma_{e,1}\sigma_{e,2}$ is the residual covariance between trait 1 and trait 2. Genetic and phenotypic correlations among traits were calculated as the covariance divided by the product of the standard deviations of the two traits.

We estimated the genetic correlation between the same traits measured on different (related) individuals in the aerated and non-aerated ponds with the bivariate Model [2.1]. The additive genetic variance-covariance matrix is the same as the bivariate model 1 where $\sigma_{A,1}^2$ is the additive genetic variance for the traits in the aerated ponds, $\sigma_{A,2}^2$ is the additive genetic variance for the traits in the non-aerated ponds and $r_{A,12}\sigma_{A,1}\sigma_{A,2}$ is the additive genetic covariance between aerated and non-aerated ponds.

The covariances of residuals between environments was set to zero, as a fish performed in only one environment. The residual variance-covariance matrix is $\begin{bmatrix} \sigma_{e,a}^2 & 0 \\ 0 & \sigma_{e,na}^2 \end{bmatrix}$ where $\sigma_{e,a}^2$ is the residual variance for the trait in the aerated ponds and $\sigma_{e,na}^2$ is the residual variance for the trait in the non-aerated ponds.

2.3 Results

We estimated the weight exponent (*f*) to be 1.77 for the fish in this experiment. The non-linear regression coefficient (b_i) obtained from Eq. [2.1], that is equivalent to the daily growth coefficient (*DGC*) per fish showed heterogeneous variances in expected weight between time points (Supplement 2.1). The estimate of the expected weight, obtained using the slope of the linear regression at the *1/f* scale and the deviations from straight-line regression on the *1/f* scale (calculated with Eq. [2.3]; Figure 2.1) reduces the heterogeneous variances of the deviations compared to the deviations at the observed scale (calculated with Eq. [2.4]; Figure 2.2) and reduces the bias of deviations, particularly at the initial and final time point of measurement, compared to the deviations at the *1/3* scale (calculated with Eq. [2.5]; Figure 2.3).

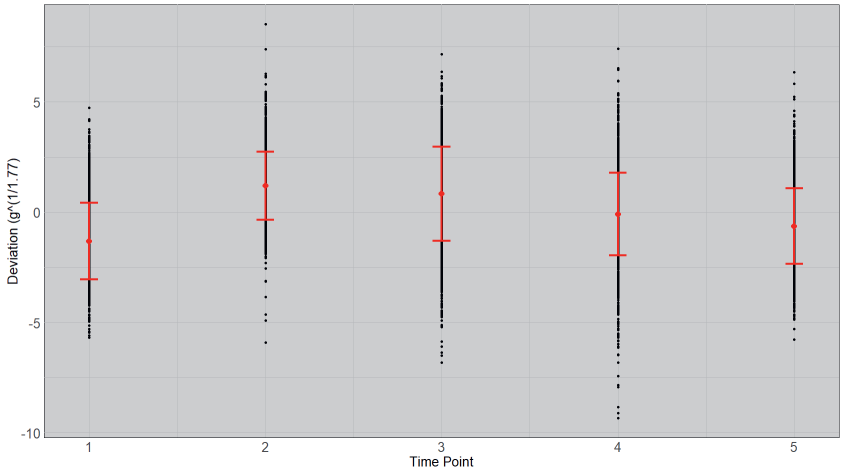

Figure 2.1 Mean and standard deviation of the weight deviation from expected individual weight (gram) with non-linear regression in *1/f* scale at stocking (t_1), three interval time points (t_{2-4}: 55, 104 and 167 days) and at harvest (t_5: 217 days) for all fish. The weight of each fish is plotted as a dot with the standard error limits shown by two short horizontal lines and the mean is located at the mid-point between these.

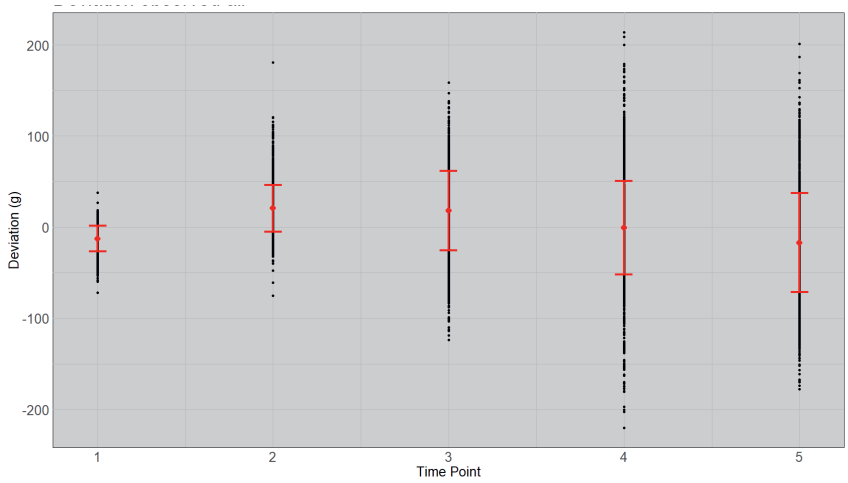

Figure 2.2 Mean and standard deviation of the observed weight deviation from expected individual weight (gram) with non-linear regression in observed *f* scale at stocking (t_1), three interval time points (t_{2-4}: 55, 104 and 167 days) and at harvest (t_5: 217 days) for all fish. The weight of each fish is plotted as a dot with the standard error limits shown by two short horizontal lines and the mean is located at the mid-point between these.

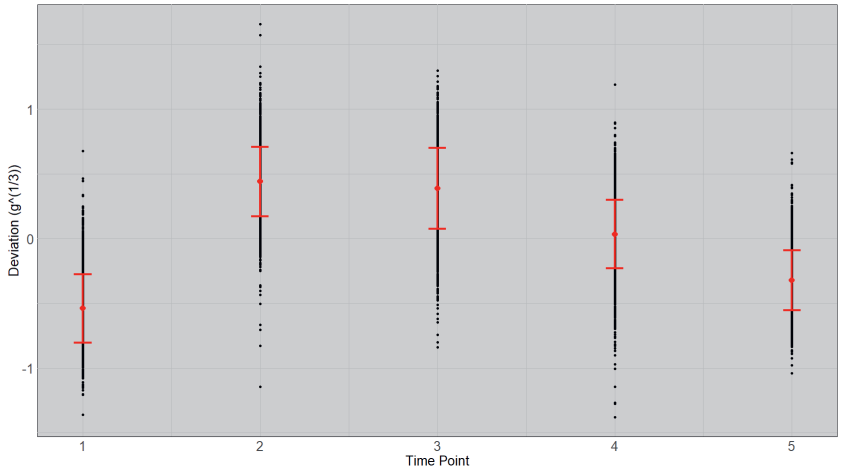

Figure 2.3 Mean and standard deviation of the standardized weight deviation from expected individual weight (gram) with cubic root transformations at stocking (t_1), three interval time points (t_{2-4}: 55, 104 and 167 days) and at harvest (t_5: 217 days) for all fish. The weight of each fish is plotted as a dot with the standard error limits shown by two short horizontal lines and the mean is located at the mid-point between these.

2.3.1 Descriptive statistics

Descriptive statistics for W_5, *DGC*, $LnVar_{ind}$ and $LnVar_{coh}$ are shown in Table 2.1. There were no significant differences for the mean $LnVar_{coh}$ between aerated and non-aerated ponds. Mean W_5, *DGC* and $LnVar_{ind}$ in the aerated ponds were significantly higher compared to non-aerated ponds ($P < 0.01$). The coefficient of variation for $LnVar_{ind}$ in the non-aerated ponds was higher compared to that for the aerated ponds.

Table 2.1 Descriptive statistics of log transformed variance from individual and cohort approach ($LnVar_{ind}$ and $LnVar_{coh}$), daily growth coefficient (DGC) and harvest weight (W_5)

	Aerated (N = 884)				Non-aerated (N = 800)			
	mean (sd)	min	max	CV	*mean (sd)*	min	max	CV
W_5 (gram)*	781.04 (265.58)	185.7	1588.6	0.34	579.87 (154.60)	135.5	1003.4	0.26
DGC ($g^{1/1.77}$/day)*	0.16 (0.04)	0.05	0.26	0.25	0.13 (0.03)	0.05	0.20	0.23
$LnVar_{ind}$*	1.33 (1.04)	-3.33	3.47	0.78	1.03 (1.26)	-3.90	3.53	1.22
$LnVar_{coh}$	-1.09 (0.98)	-5.68	1.35	0.90	-1.23 (1.03)	-5.27	2.18	0.83

N= number of observations (n) in the aerated and non-aerated ponds

* t-tests indicated that mean values were significantly different between the aerated and non-aerated pond ($P < 0.001$)

2.3.2 Genetic and phenotypic parameters

Table 2.2 shows the estimated genetic parameters of $LnVar_{ind}$, $LnVar_{coh}$, W_5 and *DGC* from the aerated and non-aerated ponds. For W_5 and *DGC*, the heritabilities were higher in the aerated ponds than the non-aerated ponds, although the means were overlapped by the standard error limits of the other value. In the non-aerated ponds, genetic variances for $LnVar_{ind}$ were more than four times higher than for $LnVar_{coh}$, and heritability estimates two times higher. In the aerated ponds the estimates were higher for $LnVar_{coh}$ than for $LnVar_{ind}$. Heritability estimates for both $LnVar_{ind}$ and $LnVar_{coh}$ were higher in the non-aerated pond (0.28 and 0.12 respectively) compared to the aerated pond (0.06 and 0.10 respectively; Table 2.2).

Table 2.2 Genetic variances (σ^2_A), phenotypic variances (σ^2_P), heritability (h^2) of log transformed variance from cohort and individual approach ($LnVar_{coh}$ and $LnVar_{ind}$), daily growth coefficient from five weight records (DGC) and harvest weight (W_5) and their standard errors (se) in the aerated and non-aerated ponds.

Trait	Aerated			Non-aerated		
	σ^2_A	σ^2_P	$h^2 \pm se$	σ^2_A	σ^2_P	$h^2 \pm se$
$LnVar_{ind}$	0.060	1.057	0.06 ± 0.03	0.440	1.563	0.28 ± 0.06
$LnVar_{coh}$	0.091	0.907	0.10 ± 0.05	0.118	0.988	0.12 ± 0.05
DGC ($g^{1/1.77}$ /day)	0.25×10^{-03}	0.88×10^{-03}	0.29 ± 0.06	0.12×10^{-03}	0.48×10^{-03}	0.25 ± 0.07
W_5 (g)	8444.79	37274	0.23 ± 0.06	2791.11	15148	0.18 ± 0.06

2.3.3 Genetic correlations

The genetic correlations between $LnVar_{ind}$, $LnVar_{coh}$, W_5 and DGC in the aerated pond are shown in Table 2.3. In the aerated ponds, the genetic correlation between $LnVar_{ind}$ and $LnVar_{coh}$ was moderate (0.46). We found moderate and positive genetic correlations between $LnVar_{coh}$ and both DGC and W_5 in the aerated ponds (0.43 and 0.35, respectively). In contrast, the genetic correlations between $LnVar_{ind}$ and DGC, as well as between $LnVar_{ind}$ and W_5 were moderate and negative (-0.44 and -0.45, respectively).

Table 2.3 Estimated genetic (above diagonal) and phenotypic (below diagonal) correlations of log transformed variance from individual and cohort approach ($LnVar_{ind}$ and $LnVar_{coh}$), daily growth coefficient (DGC) and harvest weight (W_5) of tilapia in the aerated ponds. Standard errors are in the brackets.

	$LnVar_{ind}$	$LnVar_{coh}$	DGC	W_5
$LnVar_{ind}$	X	0.46 (0.32)	-0.44 (0.23)	-0.45 (0.24)
$LnVar_{coh}$	0.32 (0.03)	X	0.43 (0.24)	0.35 (0.26)
DGC	-0.29 (0.03)	0.07 (0.04)	X	0.99 (0.00)
W_5	-0.23 (0.03)	0.10 (0.04)	0.92 (0.01)	X

Table 2.4 shows the genetic correlations between $LnVar_{ind}$, $LnVar_{coh}$, DGC and W_5 in the non-aerated ponds. In the non-aerated ponds, the genetic correlation between $LnVar_{ind}$ and $LnVar_{coh}$ was observed to be low (0.20). We estimated low genetic correlations between $LnVar_{coh}$ and both DGC and W_5 in the non-aerated ponds, (-0.06 and 0.01, respectively). The genetic correlations between $LnVar_{ind}$ and DGC and those between $LnVar_{ind}$ and W_5 were moderate and negative (-0.68 and -0.52, respectively).

Table 2.4 Estimated genetic (above diagonal) and phenotypic (below diagonal) correlations of log transformed variance from individual and cohort approach ($LnVar_{ind}$ and $LnVar_{coh}$), daily growth coefficient (DGC) and harvest weight (W_5) of tilapia in the non-aerated ponds. Standard errors are in the brackets.

	$LnVar_{ind}$	$LnVar_{coh}$	DGC	W_5
$LnVar_{ind}$	X	0.20 (0.24)	-0.68 (0.12)	-0.52 (0.17)
$LnVar_{coh}$	0.03 (0.04)	X	-0.06 (0.26)	0.01 (0.29)
DGC	-0.42 (0.03)	-0.01 (0.04)	X	0.99 (0.00)
W_5	-0.33 (0.03)	0.04 (0.04)	0.96 (0.00)	X

The genetic correlations between the aerated and non-aerated ponds for $LnVar_{ind}$ and $LnVar_{coh}$ were estimated from the bivariate model. The genetic variance for $LnVar_{ind}$ in the non-aerated ponds was higher than in the aerated ponds whereas the genetic variance for $LnVar_{coh}$ in the non-aerated ponds was comparable to the aerated ponds (Table 2.2, Figure 2.4). The genetic correlation between the aerated and non-aerated ponds was lower for $LnVar_{ind}$ (0.50 ± 0.30) than for $LnVar_{coh}$(0.80 ± 0.17, Mengistu et al., 2022). This result shows that $LnVar_{ind}$ is genetically different in both environments with a substantial degree of genotype by environment interaction (GxE).

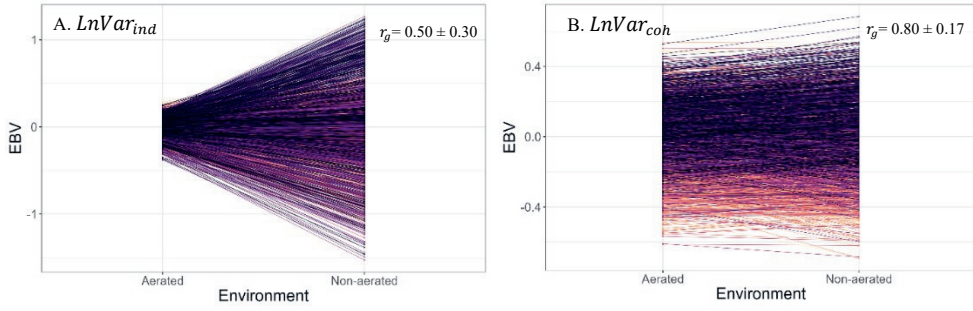

Figure 2.4. The individual estimated breeding values (EBV) for log transformed variance of deviations from (A) individual approach ($LnVar_{ind}$) and (B) cohort approach ($LnVar_{coh}$)

between the aerated and non-aerated ponds. The genetic correlations (r_g) with standard error are included inside the plot.

2.4 Discussion

This study investigated the resilience indicator *LnVar* when calculated using the individual growth curve as the expected growth performance (*LnVar*$_{ind}$). We aimed to improve the definition of *LnVar* to better capture the response of individual fish to environmental stressors. In the next part, we discuss the comparison of resilience measured with *LnVar*$_{ind}$ and *LnVar*$_{coh}$, the implications of including *LnVar*$_{ind}$ in tilapia breeding programs and the potential for further improvements to calculate *LnVar*$_{ind}$ as the indicator for resilience.

2.4.1 Definition of *LnVar*$_{ind}$

We calculated *LnVar* based on deviations from expected individual weights fitted from weight observations at five time points. To obtain the expected individual weights, we estimated the weight exponent from nonlinear regression of observed weight on the five fish ages. The nonlinear growth model uses regression parameters to describe the shape of the generated curve (Lugert et al., 2016). Our estimated growth exponent (f) of 1.77 is comparable to the growth exponent from studies by Mayer et al. (2012) and Janssen et al. (2017a) who reported weight exponents for gilthead seabream of 1.54 and 1.63, respectively. The change in fish size between time points typically leads to the heteroscedasticity. However, the calculation of variance of the deviation from straight-line regression on the 1/f scale removes heteroscedasticity. Furthermore, we fitted the weight data at the 1/f scale to both a linear model and a quadratic model and compared these two models to investigate if the use of a straight line regression is reasonable. Although the estimated regression coefficients between Models 1 and 2 are significantly different ($P < 0.05$), the slope of the quadratic term in Model 2 is very small (-0.0001648), suggesting that the quadratic term may not add much explanatory power to the model.

2.4.2 Comparison with *LnVar*$_{coh}$

We found that the heritability estimate for *LnVar*$_{ind}$ was more than two times higher than for *LnVar*$_{coh}$ in the non-aerated ponds (0.28 and 0.12, respectively). The heritability estimate of *LnVar*$_{ind}$ was also four times higher in the non-aerated pond, compared to the aerated pond (0.28 vs 0.06), while the study by Mengistu et al. (2022) showed no significant difference in the heritability for *LnVar*$_{coh}$ between non-aerated and aerated ponds (0.12 ± 0.05 and 0.10 ± 0.05, respectively). These significance differences may indicate that *LnVar*$_{ind}$ more accurately

captures the response of fish to environmental stressors than $LnVar_{coh.}$ We measured resilience using *LnVar* based on the deviations of the observed weight from the expected weight. These deviations indicated the response of an individual to the environmental change. The expected weight as the baseline to calculate the deviation should be independent from the environmental change. $LnVar_{coh}$ was calculated based on the deviation from the mean weight of the fish cohort. Changes in the mean weight of the fish cohort depend on environmental conditions between time points that affect all fish in the cohort in the same manner. If the mean weight of the fish cohort changes due to the environmental effect, the response of individual fish is relative to these "group" changes. Therefore, the calculation of $LnVar_{coh}$ using the deviation from the mean weight of the fish cohort actually estimates the residual response, as the fish cohort response is already embedded within the mean weight of the fish cohort and is not shown in the deviation used to calculate *LnVar* .The expected individual growth curve to calculate $LnVar_{ind}$ is fitted from five individual weight records and therefore, produces a smoother curve compared to the mean cohort weight that exhibits more erratic behaviour between time points. The expected individual growth curve is independent of the change in environmental conditions and able to disentangle the response of fish cohort to the environmental change. Therefore, using the expected individual growth curve to calculate the deviation in calculation of $LnVar_{ind}$ can better capture the response of fish to environmental stressors.

2.4.3 The implication of including $LnVar_{ind}$ in the tilapia breeding program

$LnVar_{ind}$ is moderately heritable in non-aerated ponds, indicating the presence of additive genetic variance for resilience in the challenging environment. The heritability for *LnVar* in our study was higher than that reported for layer chicken (0.10±0.04, Berghof et al., 2019b) and pigs (0.11±0.03, Gorssen et al., 2023). Berghof et al. (2019a) calculated *LnVar* based on the deviation from the mean weight of the cohort in chicken. Gorssen et al. (2023) used individual body weight records of pigs which were fitted with a Gompertz growth curve. The heritability estimates for $LnVar_{ind}$ are higher in non-aerated ponds than the aerated ponds. Non-aerated ponds are typical for smallholder tilapia production systems (Mengistu et al., 2022). We hypothesize that fish grown in non-aerated ponds face significant challenges due to daily recurrent hypoxia, leading to increased expression of genetic variation in *LnVar* .

Growth remains the primary trait of interest in aquaculture production and breeding programs (Chavanne et al., 2016; Houston et al., 2022). Understanding the genetic

correlation between growth and resilience is essential for optimizing breeding programs for both growth and resilience. The expected correlation between growth and *LnVar* can be explained by resource allocation theory, where energy allocation to cope with environment stressors may divert energy away from growth (Barton, Bruce A., 2002; Guderley and Pörtner, 2010), leading to decreased fish growth. However, in this study, the genetic correlations between $LnVar_{ind}$ and both *DGC* and W_5 were found to be moderately negative in both aerated (-0.44) and non-aerated ponds (-0.68). Here, a negative correlation is favourable for simultaneous improvement in *LnVar* and growth, while a positive correlation is unfavourable. Therefore, selecting for growth in the challenging environment can be expected to improve *LnVar*. Simultaneous selection for two traits often results in a negative correlation due to the action of pleiotropic genes, which affect both traits in the desired direction by selection and are rapidly brought toward fixation (Falconer, 1996). The Genetically Improved Farmed Tilapia (GIFT) strain tilapia used in this study had already undergone 17-18 generations selection for growth (Mengistu et al., 2022). The observed favourable correlation between growth rate and *LnVar* suggests that long-term selection for growth has led to increase in *LnVar*.

We observed a substantial genotype by environment interaction (GxE) for $LnVar_{ind}$, as indicated by the genetic correlation of 0.50 between the aerated and non-aerated ponds. In non-aerated ponds, a correlated response is defined as the multiplication of the genetic correlation between aerated and non-aerated ponds, the ratio of genetic standard deviations between aerated and non-aerated ponds, and the selection response in aerated ponds. When the genetic correlation is less than one, the correlated response is smaller than the direct response, assuming that the heritabilities in the two environments are similar (Falconer, 1990). Given the presence of GxE between the aerated and non-aerated ponds for $LnVar_{ind}$, it is obvious that the genetic improvement in the aerated selection environment will not be fully realized in the non-aerated production environment. If the breeding goal is to increase resilience in non-aerated production environments and selection must be conducted in an aerated nucleus, it is crucial to integrate information of own individual performance in the aerated environment with relative's records in the non-aerated environment. This integration of information could enhance selection accuracy and the genetic gain for resilience. A study by Mulder and Bijma (2005) showed that incorporating performance data from the production environment in an index significantly increases genetic gain in that environment if GxE is present.

2.4.4 $LnVar_{ind}$ as the indicator for resilience

LnVar measures the constancy of fish growth during the grow-out period. The constancy of fish growth can be an indicator for the fish's response to the stressors. As the available energy for growth at a specific moment is limited, coping with stress, including restoring homeostasis, may divert energy away from growth (Wieser et al., 1992; Wendelaar Bonga, 1997; Sadoul and Vijayan, 2016) and potentially lead to growth fluctuations. Various mechanisms for coping with environment stress, such as reducing feed intake, limiting food absorption and increasing energy allocation for maintenance processes, modify energy fluxes, all result in decreased energy allocation for growth (Wendelaar Bonga, 1997; Van Weerd and Komen, 1998; Sadoul and Vijayan, 2016). A study by Folkedal et al. (2012) showed that fish prioritize coping with the stressor through reduced feeding activity. Later, when the favourable conditions are restored, and food is available, fish compensate for the growth by temporarily accelerating somatic growth (Ali et al., 2003). Compensatory growth is characterized by an elevated growth rate from enhanced feed intake and efficiency (Won and Borski, 2013). This feeding response of fish to environmental stressors, with decreasing feed intake and compensating for growth, may lead to growth fluctuation. We hypothesize that more resilient fish can maintain their feed intake during stress period and may grow more constantly and perhaps better survive environmental stressors. However, there is limited understanding in this area and further study is needed. Selecting more resilient fish could lead to more constant growth, which plays a vital role in optimizing feeding strategies. In aquaculture practice, farmers predict feeding requirements using information on fish biomass based on the average weight of fish from periodic sampling to avoid under or overfeeding (Li et al., 2020; Rodríguez-Sánchez et al., 2018). Accurately predicting growth is essential for estimating fish feeding requirement (Bureau et al., 2008). Optimal feeding strategies improve the feed conversion ratio (FCR), which holds considerable economic value (Omasaki et al., 2017a) and reduces environmental impact (Besson et al., 2016). Gorssen et al. (2023) recently showed a moderate and positive genetic and phenotypic correlation between *LnVar* and individual biological FCR in pigs (0.33). Further study is needed to estimate the genetic and phenotypic correlation between individual biological FCR and the constancy of growth measured with *LnVar* in tilapia. The assumption that selecting resilient fish could lead to more efficient growth opens the opportunity to harness the economic benefits from the genetic improvement of *LnVar*.

2.4.5 Further improvement to calculate $LnVar_{ind}$ as indicator for resilience

The effect of environmental stressors on fish metabolism is evident, but finding evidence for effects on growth is often complex (Van Weerd and Komen, 1998; Sadoul and Vijayan, 2016). Whole-animal changes such as growth represent the tertiary response of fish to stressors, following hormonal changes and physiological adjustment, which are the primary and secondary physiological responses, respectively (Barton, Bruce A., 2002). Stressor exposure may affect fish growth via various factors, including feed intake, food absorption and maintenance energy (Wendelaar Bonga, 1997; Van Weerd and Komen, 1998; Sadoul and Vijayan, 2016). *LnVar*, as a resilience indicator, measures the constancy of fish growth in response to stressors. Understanding the biological mechanisms underlying *LnVar* as a resilience indicator is crucial, as well as confirming its relationships with factors that may influence growth, such as feed intake and feed efficiency. Furthermore, improved resilience could lead to enhanced immunity and disease resistance, as these are categorized as a tertiary response to stressors, similar to growth. Infectious diseases continue to pose a significant challenge affecting aquaculture productions (Naylor et al., 2021; Houston et al., 2022). In chickens, Berghof et al. (2019a) estimated a low genetic correlation between *LnVar* for growth and natural antibodies. However, there is limited understanding, and further research is needed to understand the relationships between *LnVar* and resilience indicators.

Less frequent records and longer intervals between measurements may be sufficient for traits like growth, which reacts more slowly to stressors than traits measuring physiological response. Mengistu et al. (2022) and our study found genetic variation in *LnVar* for growth with monthly weight measurements. However, Frequent measurement based on growth requires manual handling, which itself can induce stress in fish (Iversen et al., 2003; Fu and Yuna, 2022). Manual handling can cause physical stressors and acute stress, affecting fish behaviour, welfare and growth (Pickering et al., 1982; Ashley, 2007). Therefore, there is a need for low or non-invasive tools to enable frequent measurements. Automated phenotyping offers a non-invasive solution, making longitudinal measurements per individual fish more effortless and potentially more accurate (Li et al., 2020; Fu and Yuna, 2022). Automated phenotyping technology has been developed and applied in various aquaculture species, including salmon, catfish, tilapia and seabream (Tuckey et al., 2022; Sanchez et al., 2018; Gümüş et al., 2021; Fernandes et al., 2020; Xue et al., 2023). The evolving technology of automated phenotyping in fish will significantly facilitate the

application of $LnVar_{ind}$ as the resilience indicator in breeding programs for aquaculture species.

2.5 Conclusion

We improved the calculation of *LnVar* to better capture the response of individual fish to environmental stressors in the fluctuating environment with $LnVar_{ind}$. $LnVar_{ind}$ should be measured on 1/*f* scale to avoid heteroscedasticity. The exponent "*f*" should be estimated directly from the data. $LnVar_{ind}$ was found to be highly heritable in the more challenging environment and this can be exploited by selective breeding. The negative correlation between $LnVar_{ind}$ and growth rate implies that selection for growth may also improve *LnVar* . Whether selection for *LnVar* improves resilience and FCR remains to be tested. We recommend measuring *LnVar* through repeated weight records and based on the individual expected growth trajectories in fish breeding programs to simultaneously improve resilience and growth.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential and can be obtained by requesting WorldFish or Animal Breeding and Genomics, Wageningen University & Research (John W.M. Bastiaansen, email: john.bastiaansen@wur.nl).

Acknowledgements

This study was made possible through support provided by Indonesia Endowment Fund for Education, Ministry of Finance Indonesia (LPDP - RI) grant number 20195221414387, by the International Fund for Agricultural Development (IFAD) and by the CGIAR Research Program on Fish Agri-Food Systems (FISH) and by the CGIAR Research Initiative on Resilient Aquatic Food Systems for Healthy People and Planet, both led by WorldFish. Both these programs are supported by contributors to the CGIAR Trust Fund. We acknowledge WorldFish GIFT breeding team, and the Aquaculture Extension Centre, Department of Fisheries Malaysia, Jitra, Kedah state for their technical support throughout the project.

Supplement 2.1 The estimated expected weight (W_{exp}) using the slope of the non-linear regression (b_i) obtained from non-linear regression

The non-linear regression coefficient (b_i) obtained from Eq. [2.1], that is equivalent to the daily growth coefficient (*DGC*) per fish showed heterogeneous variances in expected weight between time points.

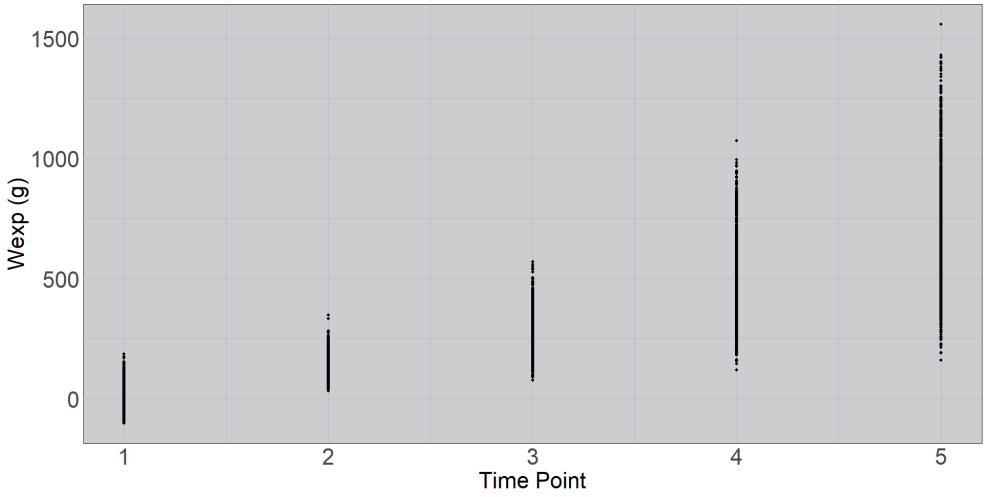

Figure 2.5 The estimated expected weight (W_{exp}) using the slope of the non-linear regression (b_i) obtained from non-linear regression (Eq 1.) at stocking (t_1), three interval time points ($t_{2\text{-}4}$: 55, 104 and 167 days) and at harvest (t_5: 217 days) for all fish

Chapter 3

The economic value of *LnVar*, a novel indicator for growth consistency in fish

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In preparation for the submission

Abstract

Fish growth is heavily influenced by water quality parameters due to the reliance on the ambient environment. Therefore, it is important for farmers to have fish that show more consistency in growth despite environmental stressors. Growth consistency can be measured using log-transformed variance of the deviations from the expected growth performance (*LnVar*), which is heritable and can be exploited through selective breeding. To determine how much emphasis to place on *LnVar* in a breeding goal, we can calculate its economic value. The economic value (EV) of *LnVar* is the extra profit at fish or farm level, generated from reducing *LnVar*. We define the economic value of *LnVar* as the effect of fluctuations in fish growth as the economic loss resulting from feed waste, growth deficiency and feed saving. In aquaculture practices, feed requirements are predicted based on expected fish weight, estimated from periodic sampling of groups of fish. It can be hypothesized that deviations of actual weight from expected weight lead to economic losses. Fish weights below the expectation will result in feed waste, while fish weights above the expectation will lead to underfeeding and reduced growth. The objectives of this study were to derive the economic value of *LnVar* and to explore the potential of economic gain from reducing *LnVar* using selective breeding. To calculate the economic value of *LnVar* , we used longitudinal records of weight gain and feed intake from GIFT tilapia that were individually reared in a recirculating system. We calculated the costs and savings during 5 time windows in the grow-out period. To calculate the economic value of *LnVar* we define the effect of fluctuations in fish growth as an economic loss resulting from feed waste, growth deficiency and feed saving. EV for *LnVar* is 0.043 US$/unit LnVar/kg. production. The breeding program to improve *HW* and *LnVar* with the selection index only on *HW* showed a total economic response of 0.110 US$/kg per generation, whereas incorporating *LnVar* into the index alongside *HW* increased the response to 0.122 US$/kg, showing approximately 11% improvement in economic response. Therefore, we recommend that fish breeding programs collect repeated records of body weight and include *LnVar* in the breeding goal.

Keywords: Economic value, variance of deviation of individual growth, tilapia, selective breeding

3.1 Introduction

Fish growth is heavily influenced by water quality parameters due to the reliance on the ambient environment (Boyd, 2017). Exposure to suboptimal water-quality parameters can induce stress in fish, leading to reduced feed intake and consequently decreased growth (Wedemeyer, 1996; Boyd, 2017; Sadoul and Vijayan, 2016; Barton, B. A., 2002; Bœuf and Payan, 2001). Therefore, it is important for farmers to have fish that show more consistent growth despite environmental stressors (Reid et al., 2019; Dabbadie et al., 2019b; Soto et al., 2018; Sae-Lim et al., 2017; Agha et al., 2018). Growth consistency can be measured using the variance of deviations between observed and expected performance from longitudinal records on body weight, known as *LnVar*. *LnVar* has moderate heritability and is easy to calculate from longitudinal weight records (Elgersma et al., 2018; Berghof et al., 2019a). Studies by Mengistu et al. (2022) and Aththar et al. (Chapter 2) showed that *LnVar* for growth of tilapia in ponds with daily fluctuations of dissolved oxygen is heritable. This suggests that *LnVar* can be exploited through selective breeding. Determining the breeding goal is the first and an important step for designing a breeding program. The breeding goal specifies the traits to be improved and their relative economic weights (Groen, 2000; Goddard, 1998). The economic values reflect the economic profit that can be obtained from genetic improvement of a trait within a production system (Groen, 2000). In aquaculture species, Janssen et al. (2017a) developed a bio-economic model to determine the economic values of growth and feed intake in gilthead seabream, while Besson et al. (2017) derived the economic value of growth and FCR in sea bass.

The economic value of *LnVar* can be determined by calculating the extra profit gained from decreasing *LnVar* or loss in profit from increasing *LnVar*. In aquaculture practice, farmers predict feed requirements using information of fish biomass, which is based on predicted weights extrapolated from periodic sampling (Li et al., 2020; Rodríguez-Sánchez et al., 2018). Fish with lower *LnVar* grow more consistently than those with higher *LnVar*. Fish with higher *LnVar* will deviate more below or above the predicted weight. We hypothesize that these deviations will lead to economic losses due to overfeeding or underfeeding because the feeding rate is calculated based on the predicted weight. Overfeeding occurs when fish weights are below the predicted average, resulting in feed waste, while underfeeding occurs when fish weights exceed the expectation, leading to reduced growth. The total economic loss per fish from increased *LnVar* therefore includes the cost of feed waste due to overfeeding, as well as the losses in growth and the savings in feed expenditure due to underfeeding. The objectives of this study were to translate these assumptions in a model to

calculate the economic value of *LnVar* and to predict the potential economic gain from selective breeding aimed at reducing *LnVar*.

3.2 Material and Methods

3.2.1 The concept of economic value of LnVar

Growth consistency, expressed as *LnVar*, is calculated from longitudinal weight measurements. At a specific time point, there are two possible deviations: 1) the observed weight is below the expected weight, or 2) the observed weight is above the expected weight. We illustrate the concept of calculating the economic value of *LnVar* in Figure 3.1. Fish A is a fish with high *LnVar* and Fish B is a fish with low *LnVar*. We assume that Fish B with low *LnVar* grows exactly according to the expected growth trajectory and Fish A with high *LnVar* grows below or above expected growth trajectory. Both of Fish A and B will be fed according to the expected growth trajectory. We assume that Fish B with low *LnVar* grows exactly according to the expected growth trajectory and Fish A with high *LnVar* grows below and above the expected growth trajectory. The dots show the weights of both fish assuming they would be fed ad libitum. However, in practice, both fish A and B will be fed according to their expected growth trajectory. Fish A will be underfed from t_1 onwards, leading to growth deficiency. However, there is also a cost reduction from feed not given (feed saved). From t_2 onwards Fish A will be overfed, leading to feed waste. In contrast, Fish B will be fed according to the predicted feeding rate based on the expected growth trajectory.

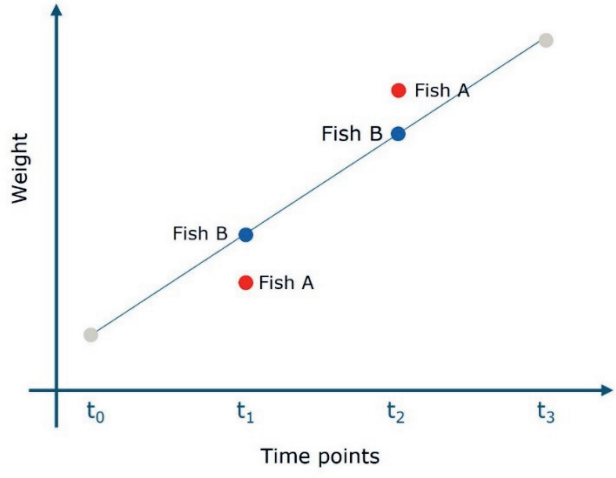

Figure 3.1 Growth trajectories for fish with high *LnVar* (Fish A) and low *LnVar* (Fish B). The grey dots at t_0 and t_3 represents the initial weight and the final weight of both Fish. The blue line represents the expected growth trajectory for both of Fish A and B and fish will be fed according to this line. The red dots represent the growth trajectory of Fish A. The blue dots represent the growth trajectory of Fish B.

3.2.1.1 Feed waste

When the observed weight is under the expected weight at time point *t*, we obtain an economic loss due to feed waste. We first calculate the observed weight gain per fish during the preceding period (*n*) as:

$$WG_{obs,in}(\text{gr}) = W_{obs,it} - W_{obs,it-1} \quad \text{Eq. [3.1]}$$

where $WG_{obs,in}$ is the observed body weight gain (in gram) for fish *i* in period *n*, $W_{obs,it}$ is observed weight for fish *i* at timepoint *t*, and $W_{obs,it-1}$ is observed weight for fish *i* at timepoint *t-1*

Then, the observed total feed required (in gram) per fish in period *n* is calculated as:

$$TF_{obs,in}(\text{gr}) = FCR_{total,i} * WG_{obs,in} \quad \text{Eq. [3.2]}$$

Where $TF_{obs,in}$ is the total feed quantity required for fish *i* in period *n*, $FCR_{total,i}$ is feed conversion ratio for fish *i* during the study period, and $WG_{obs,in}$ is the observed weight gain for fish *i* during period *n*.

We calculate expected weight gain (in gram) per fish in period n as the difference between the expected weight at age *t* and observed weight at age *t-1* as:

$$WG_{exp,in}(gr) = \left(W_{obs,it-1}^{\frac{1}{f}} + DGC_i * d\right)^f - W_{obs,it-1} \quad \text{Eq. [3.3]}$$

Where $WG_{exp,in}$ is the expected weight gain for fish *i* in period *n*, $W_{obs,it-1}$ is observed weight of fish *i* at age *t-1* , *f* is the overall weight exponent, DGC_i is the daily growth coefficient of fish *i* and *d* is the growing days.

The expected total feed quantity required (in gram) per fish in period *n* is calculated as:

$$TF_{exp,in}\ (\text{gr}) = FCR_{total,i} * WG_{exp,in} \quad \text{Eq. [3.4]}$$

Where $TF_{exp,\ in}$ is the expected total feed quantity for fish *i* in period *n*, $FCR_{total,i}$ is feed conversion ratio for fish *i* during the study period and $WG_{exp,in}$ is the expected weight gain for fish *i* in period *n*.

Finally, we calculate feed waste (in gram) per fish in period *n* as:

$$FW_{in}(\text{gr}) = TF_{exp,in} - TF_{obs,in} \quad \text{Eq. [3.5]}$$

Where FW_{in} is the feed waste for fish i in period n, $TF_{exp,in}$ and $TF_{obs,in}$ were calculated using Eq. [3.4] and [3.2], respectively.

The economic loss (in US$) due to the feed waste per fish in period n is calculated as:

$$Economic\ loss\ FW_{in}(\text{US\$}) = {}^{FW_{in}\ (\text{in gr})}/_{1000} * \ feed\ price\ (\text{in US\$/kg}) \quad \text{Eq. [3.6]}$$

Where *Economic loss* FW_{in} is economic loss due to the feed waste for fish *i* in period *n*, FW_{in} the feed waste for fish *i* in period *n* and *feed price* is tilapia feed price.

3.2.1.2 Growth deficiency

When the observed weight is above expected weight in period *n*, we obtain an economic loss due to growth deficiency. We define growth deficiency for these fish (in gram) per period as the difference between observed weight gain and expected weight gain in period *n*:

$$GD_{in}(\text{gr}) = WG_{obs,in} - WG_{exp,in} \quad \text{Eq. [3.7]}$$

Where GD_{in} is growth deficiency for fish *i* in period *n*, $WG_{obs,in}$ and $WG_{exp,in}$ were calculated using Eq. [3.1] and [3.3], respectively.

The economic loss (in US$) due to the growth deficiency per fish in period *n* is calculated as:

$$Economic\ loss\ GD_{in}(\text{US\$}) = {}^{GD_{in}\ (\text{gr})}/_{1000} * \ fish\ price\ (\text{US\$/kg}) \quad \text{Eq. [3.8]}$$

Where *Economic loss* GD_{in} is economic loss due to the growth deficiency for fish *i* in period *n*, GD_{in} is growth deficiency for fish *i* in period *n* and *fish price* is farm gate tilapia price.

3.2.1.3 Feed saved

When the observed weight is above expected weight in period *n*, we obtain an economic gain due to feed saved. We calculate feed saved for these fish (in gram) in period *n* as:

$$FS_{in}(\text{gr}) = TF_{obs,in} - TF_{exp,in} \quad \text{Eq. [3.9]}$$

Where FS_{in} is the feed saving for fish *i* in period *n*, $TF_{obs,in}$ and $TF_{exp,\ in}$ were calculated using Eq. [3.2] and [3.4], respectively.

The economic gain (in US$) due to the feed saved per fish in period *n* is calculated as:

$$Economic\ gain\ FS_{in}\ (\text{US\$}) = {}^{FS_{in}\ (\text{gr})}/_{1000} * \ feed\ price\ (\text{US\$/kg}) \quad \text{Eq. [3.10]}$$

Where *Economic gain* FS_{in} is the economic gain due to the feed saved fish *i* in period *n* and FS_{in} is the feed saved for fish *i* in period *n*.

3.2.1.4 The total economic loss

We calculate the economic loss (in US$/kg) per fish in period *n* as:

$$Economic\ loss_{in}\ (\text{US\$/kg}) = Economic\ loss\ FW_{in} + Economic\ loss\ GD_{in} - Economic\ gain\ FS_{in}$$

Eq.[3.11]

where *Economic loss*$_{in}$ is the total economic loss for fish *i* in period *n*. *Economic loss FW*$_{in}$, *Economic loss GD*$_{in}$ and *Economic gain FS*$_{in}$ were calculated using Eq [3.6], [3.8] and [3.10], respectively.

Total economic loss per individual fish (in US$/fish) for the total grow-out period is calculated as:

$$Total\ economic\ loss_i\ (\text{US\$/fish}) = \sum_{n=1}^{N} Economic\ loss_{in}$$

Eq. [3.12]

Total economic loss$_i$ is the total economic loss for fish *i* during the study period and *Economic loss*$_{in}$ is the economic loss for fish *i* in period *n* in US$/fish and *N* is the total number of periods during the study.

3.2.1.5 The economic value of LnVar

To estimate the effect of *LnVar* on *Total Economic Loss*, we fit a linear regression of *Total Economic Loss* (in US$) on *LnVar* for each fish. The slope of the regression "*b*", indicates the change in total economic loss for one fish during the study period for one extra unit of *LnVar*. Next, we calculated the economic value of *LnVar* (in US$/unit LnVar/kg) :

$$EV_{LnVar}(\text{US\$/unit LnVar/kg}) = \frac{b\ (\text{US\$/unit LnVar/fish})}{\text{The average weight gain (g/fish)}} * 1000\ \text{g/kg}$$

Eq. [3.13]

Where *b* is the economic loss for every unit *LnVar* during the study period. To calculate the effect of *LnVar* on *Total Economic Loss* per kg, we divide *b* with *The average weight gain* over the entire grow out period.

To calculate EV_{LnVar} (in US$), we refer to σ_A *LnVar* from GIFT tilapia grown in non-aerated ponds in Chapter 2. Then, we calculated *EV*$_{LnVar}$ as:

$$EV_{LnVar}\ (\text{US\$/kg}) = EV_{LnVar}\ (\text{in US\$/unit LnVar/kg}) * \sigma_A(\text{unit LnVar})$$

Eq. [3.14]

Where *EV*$_{LnVar}$ is the economic value of *LnVar* and σ_A is genetic standard deviation of *LnVar*.

3.2.2 Calculating the economic value of LnVar

To apply the concept of an economic value of *LnVar*, we used longitudinal records of tilapia weight from an experiment conducted at the Aquaculture Extension Centre, Department of Fisheries - WorldFish, Jitra, Kedah State, Malaysia. The source of the experimental fish is the Genetically Improved Farmed Tilapia (GIFT) Breeding Program, run by WorldFish in Malaysia. The details of biological material, rearing system, feed intake measurement and FCR calculation were described in Rodde et al. (2020a). Below, we summarize the experiment including data collection of body weight and FCR calculation for this study.

Forty individuals from two families (20 full-sibs from each family) were used in the experiment. The rearing system consisted of two recirculating water systems, each including 20 aquaria. Each fish was placed into a 60 L (61 × 30 × 33 cm) single plastic aquarium at 145*dph* and left for one week to acclimatize. The experiment started at 152*dph*. Commercial tilapia feed used during the experiment was *Cargill®, Starter tilapia 6113*. Throughout the experiment, fish were fed 90% of the calculated daily feed ration DFR, divided equally over two meals. Fish were fed by hand twice a day except on days of body weight measurements when fish were fed only once. The fish were fed 90% rather than 100% of the DFR in order to reduce the amount of uneaten feed and thus the time needed for counting uneaten pellets. Each fish was weighed once a week. The DFR was updated every week for each fish. Every day, feed given to the fish was weighed and the uneaten pellets were counted and removed from the aquaria at least two hours after the last meal of the day. Daily feed intake (DFI) was calculated for each fish as the difference between daily feed weight given and daily feed weight uneaten. In this experiment, Rodde et al. (2020a) calculated feed intake (FI) and weight gain (WG) for individual fish on two week time steps (biweekly).

3.2.2.1 Feed intake, growth and *LnVar*

Rodde et al. (2020a) conducted the individual feeding experiment for GIFT strain tilapia from 152 – 362*dph*. Here we use weight and feed intake records from 208 - 278 *dph* (Figure 3.2). Based on the information from Rodde et al. (2020a), fish were not affected by sexual maturation during this period. We used biweekly weight records at 6 time points (t = 1-6) as $W_{obs,\,t}$ and feed intake records during 5 two-week interval periods (n = 1- 5) as $FI_{obs,\,n}$.

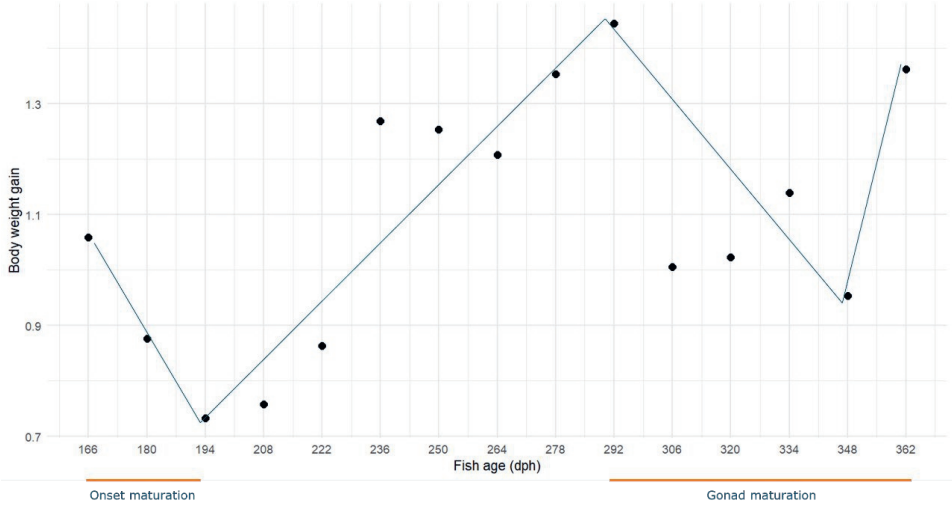

Figure 3.2 Body weight gain (gr/day) of Nile tilapia during the experiment (dots), with segmented linear regressions associated (regression lines were extended until intersection - blue line), the orange lines below the fish age indicate the onset maturation and gonad maturation. dph: days post hatching. Figure was reproduced from Rodde et al. (2020a).

We used biweekly weight records from this experiment to calculate the observed weight gain during each period (*n*) ($WG_{obs,\ in}$) in Eq. [3.1]. Then, we used biweekly feed intake records from this experiment to calculate total feed intake for each fish. We sum observed weight gain for each fish and calculate observed feed conversion ratio during the study period. The total feed intake per fish during the study period ($FI_{total,i}$) is calculated as:

$$FI_{total,i} = \sum_{n=1}^{5} FI_{obs,in} \qquad \text{Eq. [3.15]}$$

Where $FI_{total,i}$ is total feed intake for fish *i* during the study period and $FI_{obs,in}$ is feed intake for fish i in period *n*.

Next, we used weight records at t_1 and t_6 to calculate observed weight gain for fish *i* during the study period ($WG_{obs\ total,i}$) as:

$$WG_{obs\ total,i} = W_{obs,i6} - W_{obs,i1} \qquad \text{Eq. [3.16]}$$

Where $WG_{obs\ total,i}$ is the observed weight gain for fish *i* during the study period, $W_{obs,i6}$ is the observed weight of fish *i* at time point 6 and $W_{obs,i1}$ is the observed weight of fish *i* at time point 1.

Finally, we calculate total observed feed conversion ratio for fish *i* during the study period ($FCR_{total,i}$):

$$FCR_{total,i} = {FI_{total,i}}/{WG_{obs\ total,i}} \quad \text{Eq. [3.17]}$$

where $FCR_{total,i}$ is feed conversion ratio for fish *i* during the study period, $FI_{total,\ i}$ is observed feed intake for fish *i* during the study period, $WG_{obs\ total,i}$ is observed weight gain for fish *i* during the study period. We used $FCR_{total,i}$ to calculate the observed total feed required for fish *i* in period *n* ($TF_{obs,in}$) with Eq. [3.2] and the expected total feed required for fish *i* in period *n* ($TF_{exp,in}$) with Eq. [3.4].

We calculated *LnVar* with the deviations of observed weight from expected individual weight (Aththar et al., Chapter 2) at timepoints W_1 to W_6. We calculate the expected weight of individual fish *i* at age *d* as:

$$W_{exp,id}^{\frac{1}{f}} = a + DGC_i * d \quad \text{Eq. [3.18]}$$

where $W_{exp,id}$ is the expected weight of fish *i* at age *d, f* is the overall weight exponent, *a* is the intercept, DGC_i is daily growth coefficient of fish *i* and *d* is fish age. To estimate *f*, we fitted an exponential curve to the observed weights of all the fish:

$$W_{obs,id} = a + b_i * d^f \quad \text{Eq. [3.19]}$$

Where $W_{obs,\ id}$ is the weight of fish *i* at age *d*, *a* is the intercept, b_i is the slope of the non-linear regression for fish *i*, *d* is the fish age and *f* is the overall weight exponent. The weight exponent *f* was estimated using the *nls* function in R (RStudio-Team, 2022). Then, to calculate *DGC* for fish *i* (DGC_i) in Eq. [3.18], we transformed the 6 observed weights per fish as $W_{obs,id}^{\frac{1}{f}}$ to linearize the growth curve and we estimate DGC_i as the slope of the linear regression of $W^{\frac{1}{f}}$ on the age of the fish *i* at 6 time points.

Per fish, we then calculate the deviations (dev_{it}) as:

$$dev_{it} = W_{obs,it}^{\frac{1}{f}} - W_{exp,it}^{\frac{1}{f}} \quad \text{Eq. [3.20]}$$

Where dev_{it} is the deviation of observed weight from expected weight of fish *i* at time point *t*, $W_{obs,it}$ is the observed body weight of fish *i* at time point *t* and $W_{exp,it}$ is the expected body weight of fish *i* at time point *t*. Next, for each fish, we calculated the variance of the resulting 6 deviations (*Var-dev*). Finally, we log-transformed *Var-dev* using the natural logarithm to obtain log-transformed variance (*LnVar*), which is the commonly used scale to express genetic variation in residual variance (Hill and Mulder, 2010).

3.2.2.2 Economic value of *LnVar*

We used the feed price and farm gate fish price from Setyawan et al. (2022b) to calculate *Economic loss* FW_{in} with Eq. [3.6], *Economic loss* GD_{in} in Eq. [3.8] and *Economic gain* FS_{in} with Eq. [3.10]. Genetic parameters of *LnVar* estimated in Chapter 2 of this thesis were used to calculate the economic value of improving *LnVar* with one standard deviation (EV_{LnVar} in US$/kg) in Eq. [3.14] (Table 3.1).

Table 3.1 Farm gate tilapia price, tilapia feed price and h^2 *LnVar* for the calculation of economic value of *LnVar* (EV_{LnVar})

	Value	Unit
Tilapia feed price [a]	0.7	US$/kg
Farm gate tilapia price [a]	1.4	US$/kg
σ_P^2 *LnVar*[b]	1.563	-
σ_A^2 *LnVar*[b]	0.440	-
h^2 *LnVar*[b]	0.28	-

[a] Setyawan et al. (2022b), [b] Aththar et al. (Chapter 2)

3.3 Result

3.3.1 Descriptive statistics

Descriptive statistics for W_1 to W_6, *LnVar* and *DGC* are shown in Table 3.2. We estimated the overall weight exponent (*f*) to be 2.05 for Nile tilapia in this experiment. The positive values for feed waste (*FW*) and growth deficiency (*GD*) indicate a positive contribution to the economic loss, while the positive value for feed saved (*FS*) indicates a negative contribution to the economic loss.

Table 3.2 Descriptive statistics of observed weight at time point 1 - 6 ($W_{obs\ 1\text{-}6}$; in gram), log transformed variance (*LnVar*), daily growth coefficient (*DGC* ; $g^{1/2.05}$/day), feed waste (*FW*; in gram), growth deficiency (*GD*; in gram) and feed saved (*FS*; in gram) from 6 bi-weekly records.

	mean (sd)	min	max
$W_{obs\ 1}$	84.1 (22.3)	46.4	139.1
$W_{obs\ 2}$	96.2 (26.8)	51.2	170.5
$W_{obs\ 3}$	114.0 (32.1)	60.1	199.5
$W_{obs\ 4}$	131.6 (36.7)	67.1	225.5

$W_{obs\ 5}$	148.1 (41.2)	74.4	242.3
$W_{obs\ 6}$	166.5 (45.8)	82.5	264.6
LnVar	-4.62 (0.92)	-7.89	-2.84
DGC	0.049 (0.013)	0.02	0.08
FW	10.0 (6.8)	1.2	29.4
GD	5.5 (3.7)	0.4	17.4
FS	8.3 (8.1)	0.4	29.9

3.3.2 The economic value of LnVar

LnVar is positively correlated with total economic loss per fish during the grow out period (Figure 3.3). The effect of *LnVar* on *Total economic loss* during the growout period of 10 weeks is 0.004 US$. The coefficient indicates the expected increase of total economic loss for every additional unit in *LnVar* or the expected decrease of total economic loss for every reduction of *LnVar* by one unit.

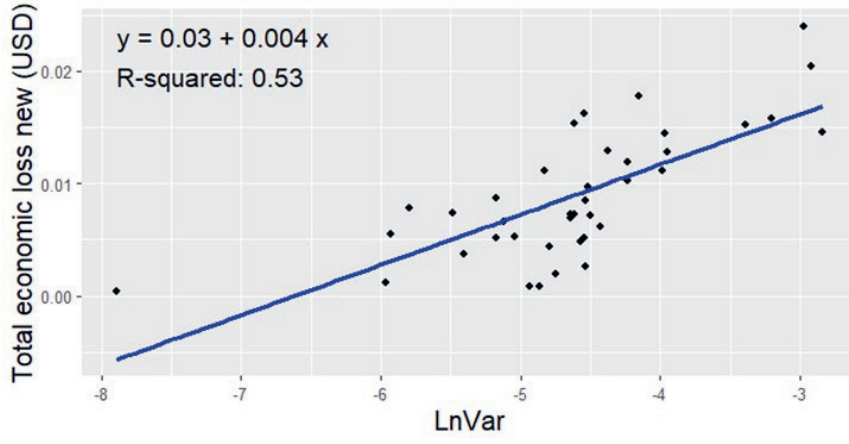

Figure 3.3. Regression of total economic loss (US$) during grow-out on *LnVar*. Dots represent individual fish.

The average weight gain in 70 days is 92.94g. The economic value of *LnVar* (EV_{LnVar}) in in US$/unit LnVar/kg is:

$$EV_{LnVar}(\text{in US\$/unit LnVar/kg}) = \frac{0.004\ \text{US\$/unit LnVar/fish}}{92.94\ \text{g/fish}} * 1000\text{g/kg} = 0.043\ \text{US\$/unit LnVar/kg}$$

Using the estimate of σ_A from Table 2.2 (Chapter 2), the economic value of improving *LnVar* with one standard deviation (EV_{LnVar} in US$/kg) is:

$$EV_{LnVar} \text{ (in US\$/kg)} = 0.043 \text{ US\$/unit LnVar/kg} * 0.42 \text{ unit LnVar} = 0.018 \text{ US\$/kg}$$

3.4 Discussion

We identified three economic effects of fluctuations in growth. First, feed waste represents the economic loss due to giving more feed than fish can use for growth. The feed requirements are calculated based on the weight at the last periodic sampling and the predicted growth rate from the known DGC. However, when fish grow less than expected, the feed given on subsequent days is more than what is needed, resulting in unutilized feed. The value of the unutilized feed is calculated as an economic loss. Second, growth deficiency represents the economic loss due to fish growing less than their potential. When the observed weight of experimental fish was above the expected weight, we assumed that this "extra" fish growth was facilitated by adjusting the feeding rate weekly during the preceding period (Rodde). In practical circumstances, the fish would not be weighed weekly and the feeding would have remained at the lower rate, based on the observed weight and DGC at the previous time point. In this case the feed for the extra growth would not have been available to the fish, making it grow slower than its potential. This difference in growth was considered an economic loss due to a growth deficiency. This growth deficiency will affect harvest weight, even when the fish grows to its full potential during the subsequent growing periods. Third, feeding the fish at the lower rate results in an economic gain due to feed saving. Feed that is not given, because the feed requirement is not adjusted weekly to the fish weight, does not have to be paid for. Feed waste and growth deficiency contribute to the economic loss. On the other hand, feed saving reduces the economic loss. Thus, the total economic loss is the sum of the three values. In this paper we developed a set of equations that quantify these processes. The resulting economic value of improving *LnVar* with one genetic standard deviation was 0.018 US$/kg. This shows that high *LnVar* can lead to economic loss and vice versa, that reducing *LnVar* will improve economic returns.

We used the dataset from an experiment by Rodde et al. (2020a), which recorded weight and feed intake of individually reared Nile tilapia, to calculate the economic value of *LnVar*. We used records between 208 and 278 days post-hatch (*dph*), a period when fish were not influenced by sexual maturation. The full individual feeding experiment was conducted from 152 to 362 *dph*. Early in the experiment, at 152 and 194 *dph*, a decrease in body weight gain among Nile tilapia was observed (Figure 3.2), which may be explained by the onset of sexual

maturation (Rodde et al., 2020a). A second decrease in body weight gain occurred between 292 and 348 *dph*. Rodde et al. (2020a) suggested that pheromones from the few females in the same water system could have been transmitted through water exchanges between tanks, potentially inducing gonad development in male tilapia. According to the dynamic energy budget (DEB) theory, energy reserves are allocated between structural growth and maturity, with a fixed fraction reserved for maintenance (Kooijman, 2010). During the onset of maturity and gonad development, an increased allocation of energy towards maturity can reduce resources available for growth, potentially leading to decreased overall growth (as reviewed by Wootton, 1985). With *LnVar* we aim to measure deviations from the expected growth trajectory that are caused by environmental stressors. Therefore the early and later timepoint before day 208 and after 278 were excluded because they were potentially also affected by maturation and gonad development.

We estimated the weight exponent for the study population from non-linear regression of observed weights at 6 time points. The estimated growth exponent (*f*) of 2.05 in this study indicates that the experimental GIFT tilapia exhibited a more linear growth curve compared to the GIFT tilapia we studied previously, which showed growth exponent of 1.77 (Aththar et al., Chapter 2). The experimental settings between these studies could explain the difference in growth exponent. In this study, GIFT tilapia were reared in recirculation water system with constant aeration system, while in the previous study, GIFT tilapia were reared in non-aerated ponds (Mengistu et al., 2020a).

To calculate feed waste we made use of the estimated individual FCR_{total} which is estimated based on the total weight gain and the total feed given over the studied period from day 208 to day 278. This provides the best estimate of the true FCR for the individual fish which we assume is staying the same over the study period. Observed FCR varies between the different growth periods, due to overfeeding and growth deficiency. The FCR during our study from 208-278 *dph* was 1.66 and comparable with the reported FCR values of GIFT tilapia grown in aerated ponds (1.73, Mengistu et al., 2020a).

Fish breeding programs typically prioritize production-related traits such as harvest weight (Houston et al., 2022; Janssen et al., 2017a; Chavanne et al., 2016), rather than focusing on *LnVar* . With a favourable correlation between *LnVar* and harvest weight (Aththar et al., Chapter 2), we expect a favourable correlated response in harvest weight when selecting for *LnVar* and vice versa. To investigate the economic response of measuring *LnVar* in addition to *HW* in a breeding program to improve *HW* and *LnVar* , we simulated two scenarios: one

with selection index only on *HW* and another with index that included *HW* and *LnVar* (Supplement 3.1). The EV for *LnVar* is 0.043 US$/unit LnVar/kg production. The EV of harvest weight (*HW*) was estimated to be 2.21 US$/kg/kg production using a general profit equation developed by Jansen et al., 2017 (Supplement 3.1). A breeding program with a selection index only on *HW* resulted in a total economic response of 0.110 US$/kg production per generation, while including *LnVar* in the selection index increased the total economic response to 0.122 US$/kg. This result shows that the effect of measuring *LnVar* and incorporating it in the selection index could lead to approximately 11% increase in economic response.

These estimates in economic responses should be treated with caution, as our economic values for *LnVar* were derived from an experimental setting which may not reflect the growth and feed intake under commercial conditions in ponds. Feed intake measured in individual rearing differs from measurements taken in groups (de Verdal et al., 2018), as the lack of social interactions between fish can potentially impact feed efficiency (Rodde et al., 2020b). However, the FCR observed during the total experiment period (1.82; Rodde et al., 2020a) and during our study period of 208-278 dph (1.66) are similar to the FCR for GIFT tilapia grown in a freshwater aerated pond (1.73, Mengistu et al., 2020a). Furthermore, individual rearing in a recirculating system in this study was designed to optimize water quality parameters for the fish, reducing the potential of stressors during the experiment. Consequently, *LnVar* was expected to be minimal, as it measures growth consistency in response to stressors. However, individual rearing may still induce stress due to social and human-induced factors, including isolation from group interaction, stressors from removing uneaten pellets and handling for weighing (Rodde et al., 2020a). We found that the range of *LnVar* in this study (-7.89 to -2.84) is lower than that observed in GIFT tilapia grown in non-aerated ponds (-3.90 to 3.53) (Aththar et al., Chapter 2).

However, if we assume that the slope of the regression between total economic loss (US$) during grow-out and *LnVar* in GIFT tilapia grown in non-aerated ponds is the same as in our current study, then the difference in the range of *LnVar* will not affect the calculated EV of *LnVar*.

It is also important to note that our economic values for EV of *HW* and *LnVar* were based on tilapia feed prices and market prices that may not reflect the current situation. However, the feed price referenced in this study (0.7 US$/kg) is comparable to the updated prices as of December 2023 with 0.64-0.75 US$/kg (Arifianto, 2023). For tilapia prices, we used the farm

gate tilapia price of 1.4 US$/kg, which falls within the lowest range of the updated prices as of December 2023 (1.30 – 2.00 US$/kg) (Arifianto, 2023). Our results nevertheless show that economic benefit could be obtained from measuring *LnVar*.

3.5 Conclusion

The economic value of LnVar quantifies the effect of fluctuations in fish growth (high or low *LnVar*) on the economic loss from three economic effects of feed waste, growth deficiency and feed saving. We found that decreasing *LnVar* will lead to a reduction in economic loss. The economic value of *LnVar* is 0.043 US$/unit LnVar/kg production. A breeding program to improve *HW* and *LnVar* with only *HW* in the selection index showed a total economic response of 0.110 US$/kg per generation, whereas adding *LnVar* into the index increased the response to 0.122 US$/kg, showing approximately 11% improvement in economic response. Therefore, we recommend that fish breeding programs collect repeated records of body weight and include *LnVar* in the selection index alongside HW in the breeding goal to improve *LnVar* and weight, which will enhance economic response.

Acknowledgements

We acknowledge Charles Rodde and Hugues de Verdal from CIRAD, France, for conducting the experiment and providing the data used in this manuscript. This study was made possible through support provided by Indonesia Endowment Fund for Education, Ministry of Finance Indonesia (LPDP - RI) grant number 20195221414387.

Supplement 3.1. Selection for harvest weight and LnVar

We compared the selection response in trait and economic units for two scenarios of tilapia breeding program, using selection indices based only on harvest weight (*HW*) or on both *HW* and *LnVar*. The selection response in trait and economic units following discrete one-stage selection were predicted using SelAction (Rutten et al., 2002). For simulation, the genetic parameters for GIFT strain tilapia in non-aerated ponds were used (Table 2.2, Chapter 2). In the breeding program, 120 females were each mated to one of 40 males (3:1 ratio) to create 120 full-sib families. 40 fish (20 females and 20 males) were kept per family (4,800 fish in total) as selection candidates. The EV of *HW* was calculated using equation 22 from Janssen et al. (2017a):

$$EV_{HW}(\text{in US\$/kg}) = \frac{\frac{1000}{HW^2} * \left(\frac{CFI * feed\ price}{0.5 + surv/200} + \frac{seed\ price}{surv/100}\right)}{Q}\ x\ 1000\frac{g}{kg}$$

Where *HW* is harvest weight, *CFI* is cumulative feed intake, *surv* is survival rate and *Q* is unit of per kg fish production. Feed price and seed price were 0.0007 US$/g and 0.004 US$/pc, respectively (Setyawan et al., 2022b). *CFI* is calculated as the function of FCR multiply by the harvest weight. To calculate *CFI* , we refer to Mengistu et al. (2020a) for FCR of 1.73 and *HW* of 580 g for tilapia. *CFI* is 1003.4 g. Further, we assumed that *surv* of GIFT strain tilapia is 90%. The value of *Q* is 1 kg fish production.

The EV of *HW* is 2.21 US$/kg/kg production, while the EV for *LnVar* is 0.043 US$/unit LnVar/kg production (this study). The scenario of breeding program to improve *HW* and *LnVar* with selection index only on *HW* (H_2) resulted in a total economic response of 0.110 US$/kg per generation, while including *LnVar* in the selection index with *HW* (H_1) increased the total economic response to 0.122 US$/kg (Table 3.3).

Table 3.3 The selection response in trait and economic unit for *HW* and *LnVar* of selection for the difference breeding goal of GIFT strain tilapia breeding program

Breeding goal	Index	Trait units		Economic units		
		HW	*LnVar*	*HW*	*LnVar*	total
$H_1 = W_{HW} * A_{HW} + W_{LnVar} * A_{LnVar}$	*HW* and *LnVar*	0.045	-0.517	0.100	0.022	0.122
$H_2 = W_{HW} * A_{HW} + W_{LnVar} * A_{LnVar}$	*HW*	0.045	-0.255*	0.099	0.011	0.110

For all the traits in selection index, we used own performance. *Correlated response in *LnVar* from selection on *HW*.

Chapter 4

Genetic parameters and genotype by environment interaction in a unique Indonesian hybrid tilapia strain selected for production in brackish water pond

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Aquaculture
DOI: https://doi.org/10.1016/j.aquaculture.2022.738626

Abstract

Recurrent farming failures due to disease outbreaks have driven Indonesian shrimp farmers to develop co-culture between shrimp and tilapia. For this reason the Research Institute for Fish Breeding (RIFB) Indonesia has started to develop a fast-growing tilapia with good growth over a range of fluctuating salinities in brackish water ponds. A freshwater nucleus and evaluation breeding program is the simplest strategy to implement, but requires knowledge on the extent of genotype by environment (GxE) interaction between fresh and brackish water environments. The objectives of this study were: 1) to investigate the presence of GxE between brackish water and freshwater ponds, 2) to investigate the impact of salinity on genetic parameters, and 3) to investigate gonadal development of tilapia in both environments. We produced 91 fish families and for each family, randomly choose 2 groups of 20 fingerlings for communal grow-out in brackish water at salinity 6 to 25 ppt and freshwater for 120-147 days. We recorded harvest weight (*HW*) and standard length (*L*) and calculated daily growth coefficient (*DGC*), growth rate in length (*GR(L)*) and condition factor (*K*) for each fish. Gonadosomatic index and maturation score (0/1) was recorded on 6 fish from each family per environment. We estimated genetic parameters using bivariate animal models in ASReml version 4.1. Results: *HW*, *L*, *DGC* and *GR* (L) in brackish water were significantly higher than in freshwater. Heritability was moderate for all traits in both environments (0.35-0.50). Genetic correlations between brackish water and freshwater for *HW*, *SL*, *DGC* and *GR(L)* were 0.65-0.74. Gonad weight for males and females, and gonadosomatic index for females in brackish water were significantly higher than in freshwater ($P<0.05$). Gonad maturity for both sexes had low heritability in brackish water than in freshwater, (0.12 and 0.04 respectively) with a genetic correlation of 0.47. We conclude that there is substantial GxE interaction for growth between brackish water and freshwater. However, the higher mean growth in brackish water suggests that this is not due to salinity per se, but more likely to other differences between the pond environments. We recommend that a breeding program for salinity tolerant tilapia with a safe, stable, low-risk, and bio-secure fresh water nucleus should incorporate sib information on growth performance in brackish water.

Keywords: breeding program, brackish water, salinity, heritability, genotype by environment interaction

4.1 Introduction

The Indonesian shrimp industry consists of approximately 65% small-scale farmers who have been abandoning their ponds in many areas due to repeated crop failures, and these reductions in production will likely accelerate as climate change drives significant changes in salinity and sea level rises (Dabbadie et al., 2019a; Kalikoski et al., 2018; Maulu et al., 2021). Because shrimp production is the most important aquaculture industry in Indonesia with the highest contribution to the national income MMAF, 2018, this has important economic and societal repercussions.

To address this situation, many shrimp farmers have shifted to a shrimp and tilapia co-culture farming system in brackish water ponds. Most tilapia grow quickly in freshwater, but some species and strains can also be cultivated in brackish water (Cnaani and Hulata, 2011; Stickney, 1986; Suresh and Lin, 1992). In addition, several studies (Aththar and Gustiano, 2010; Putra et al., 2013) demonstrated that hybrids and improved strains have higher growth than Nile tilapia in brackish water ponds (Aliah, 2017; Setyawan et al., 2015).

To facilitate this transition, the Research Institute for Fish Breeding (RIFB) has been conducting a small-scale breeding program for salinity tolerant tilapia for four generations. The institute acquired a tilapia strain from a private feed company in 2007 that was thought to be blue tilapia (*Oreochromis aureus*). The founders were spawned for multiplication in early 2008 before the start of the breeding program and named the Sukamandi strain. However, Yu et al. (2022) recently compared the whole genome sequence of this strain to both Nile tilapia (*Oreochromis niloticus*) and blue tilapia and discovered that it is actually a hybrid that is genetically closer to Nile tilapia than to blue tilapia. Signatures of introgression suggest that specific genes related to salinity adaptation (*slc25a24* and *cdhl*) have been introgressed from blue tilapia. We assume that inadvertent mixing between blue tilapia and Nile tilapia occurred between 2008 and 2011 because in subsequent communication with the feed company, they explained that they kept blue tilapia in a separate closed facility. Although this was unintentional, it has apparently contributed to improving salinity tolerance in the Sukamandi strain by introducing favourable genetic variants to the population.

To date, the Research Institute for Fish Breeding's breeding program for salinity tolerant tilapia has been based entirely on mass selection using own performance records of candidates from the Sukamandi strain evaluated in a range of brackish water environments. The average selection response after four generations of phenotypic selection for increased

harvest weight after ~120 days of growth is 10.29% for males and 9.29% for females (unpublished results).

The current strategy exposes selection candidates to conditions with poor biosecurity, high mortality losses and associated risks. Many practical challenges such as limited control over environmental conditions and transportation from test sites to the hatchery have also resulted in high risk and costs. An alternative strategy is a nucleus-based breeding program similar (Bentsen and Gjerde, 1994; Olesen et al., 2015), to those previously implemented for other tilapia strains (Omasaki et al., 2017b), salmonids (Yáñez et al., 2014), and gilthead seabream Janssen et al., 2018. This approach maintains all selection candidates in a safe and bio-secure environment that does not represent commercial growing conditions and selects among them using performance information from relatives grown in a production environment using mixed-model BLUP to estimate their breeding values (Trong, 2013).

In this case, the population of selection candidates can be kept in safer and more stable freshwater conditions at the research institute and their progeny and/or sibs can be grown and evaluated in brackish water test locations. Because genotypes may perform differently in the holding vs. testing environments due to genotype by environment interaction (Falconer and Mackay, 1996) the performance of candidates in freshwater may not predict their performance in brackish water. Depending on the strength of GxE, this approach may require predicting the breeding values of candidates based on the performance of their relatives rather than own records. GxE interaction between freshwater and brackish water has been studied previously by Luan et al. (2008b), Thodesen et al. (2011) and Thoa et al. (2016) based on the genetic correlation between final weight in different environments using models that treat them as separate traits. Thoa et al. (2016), for example, estimated the genetic correlation between harvest weight in freshwater and brackish water (15-20 ppt salinity) as 0.92 ± 0.04 which suggests that selection based on freshwater performance can transfer 92% of the genetic gain achieved to brackish water performance without evaluating sibs or other relatives in the brackish water environment. If this is also true for the Sukamandi strain, it may not be worthwhile to evaluate sib or progeny performance in brackish water.

The previous focused on final weight, but growth rate during on-growing period is the main priority for fish farmers. Selecting on fast-growth is preferable to selecting on harvest weight because it increases feed efficiency, which further contributes to profitability and sustainability of production (Aubin et al., 2009; Besson et al., 2016; de Verdal et al., 2018).

However, the genetic correlations between final weight and growth rate were greater than 0.97 (de Oliveira et al., 2016). Consequently, selection for harvest weight within a batch of selection candidates will improve growth rate as a correlated response. In this study, we directly quantify growth rate, which can be expressed as absolute or specific growth rate (Hopkins, 1992), thermal growth coefficient (Jobling, 2003) or daily growth coefficient (Cho, 1992). We expected that higher salinity would reduce growth rates and the expression of genetic variation resulting in lower genetic variance as well as re-ranking of candidates (GxE) between brackish water and freshwater. We also address the implications of GxE for breeding program to improve growth of tilapia in brackish water.

Selection for improved performance in brackish water also raises concerns about the potential for increased reproduction and higher probability that escapees may become invasive in estuarine ecosystems via correlated responses to selection. Therefore, we would ideally select for fast-growth, but lower reproduction performance in brackish water without compromising reproductive performance in the freshwater nucleus. This requires further knowledge on the correlation structure between growth and reproduction in both fresh and brackish water.

4.2 Material and Methods

4.2.1 Selected parents

We produced our experimental fish using the 4th generation of the Sukamandi strain as selected parents at the Research Institute for Fish Breeding, Indonesia. We maintained the parents in separate 15 m2 hapas (5 x 3m), cage-like, rectangular nets with a mesh size of 5 mm suspended in 2000 m2 freshwater ponds in single sex groups. They were fed twice a day on a commercial pelleted feed with approximately 30% crude protein and 5% fat, at a daily feeding rate of 3% of biomass for four weeks.

4.2.2 Family production

We produced full- and half-sib families in 65 smaller breeding hapas (4 m2; 2 × 2m), suspended in three 200 m2 earthen ponds at the Research Institute for Fish Breeding. Each of these hapas was stocked with one male and three females by introducing the males to the hapas 1 day before the 3 females. Because tilapia are mouth-breeders in which the female keeps fertilized eggs in her mouth until hatching, and there was only one male in each breeding hapa, this method produces full-sib families from each mated female and paternal half-sib families if a male mates with multiple females.

We conducted this mating process in 7 day cycles, and if none of the females produced eggs during a cycle, we replaced the male. We replaced the male in every hapa after 2 cycles, and replaced spawned females with new females. At the end of each cycle, we collected the fertilized eggs or hatchling/swim-up fry from the females' mouths, recorded her unique identification tag number, and subsequently incubated the eggs from each female in a single cone-shaped hatching jar (25 cm diameter and 40 cm height) with a constant flow of water until they hatched and grew into functional hatchling/ swim-up fry. We labelled the cone based on the female ID and recorded the collection date of eggs or larva and the male parent for each female's progeny. During this incubation period, we removed dead eggs and fry daily. We also stabilised the water temperature during incubation between 28°C and 30°C with aquarium water heaters.

In total, we produced 91 families over a period of 105 days (from 21 May to 22 August 2019) consisted of 53 full sib families and 38 paternal half-sib families. In order to facilitate the next steps of the experiment, we divided the resulting families into three batches based on the spawning date. We labelled the first 35 full-sib families from the first four weeks of the reproduction period as batch 1, the next 27 families as batch 2, and the last 29 families as batch 3.

4.2.3 Fingerling Nursery Rearing and Tagging

Fry hatched after about 5–7 days. After yolk-sac absorption, we transferred swim-up fry from each family into 4 m2 nursery hapas (2 x 2, mesh size 1 mm) suspended in a 2,000 m2 earthen pond. For this, we randomly sub-sampled 200 fry and stocked them into a single nursery hapa, equivalent to a nursing density of 50 fish per m2. During this period, we fed them twice daily using a commercial powder feed with a dietary protein level of 30%, at the rate of 10-15% of their body weight during the first 3 to 4 weeks. The second nursery period continued until tagging at an average bodyweight of 16g during which we fed the fingerlings a commercial pelleted feed consisting of 30% protein twice daily at the rate of 10% of total body weight. This nursery period of separate family rearing in nursery hapas ranged from 120 to 161 days. At the end of this period, we randomly chose and tagged 40 individuals from each family using PIT (Passive Integrated Transponder) tags and recorded their identification number, stocking weight (*SW*) and standard length (*L*).

A total 20 fingerlings per family were grown in brackish water and another 20 in freshwater. For logistical reasons, we tagged the fish for brackish water grow out first. The first batch of

35 families were tagged at an average age of 142 days for brackish water grow out and 148 days for freshwater grow out (127 to 161 days of age post-hatching interval) as summarized in Table 4.1. We tagged the second batch at the average age 134 and 141 days for brackish water and freshwater respectively (123 to 161 days of age post-hatching interval), and the third batch at the average age of 139 for brackish water and 142 for freshwater (128 to 151 days of age post-hatching interval). We then pooled all tagged fingerlings within a batch and water treatment after 3 days of conditioning in fiberglass tanks with minimum feeding rate of 1-3% body weight.

Table 4.1 Total number of families, range (mean) of stocking age and harvesting age and rearing period in days, stocked number and harvested number of fish in brackish water (B) and freshwater (F)

Batch	Environment	Number of families	Stocking age	Harvest age	Number stocked	Number Harvested	Rearing period
1	B	35	127-155 (142.6)	253-281 (268.6)	640	511	126
	F	35	133-161 (148.6)	258-286 (273.6)	649	482	125
2	B	27	123-154 (134.9)	243-274 (254.9)	489	348	120
	F	27	130-161 (141.9)	270-301 (281.9)	488	425	140
3	B	29	128-148 (139.4)	250-270 (261.4)	568	449	122
	F	29	131-151 (142.4)	278-298 (289.4)	574	484	147
All	B	91	123-155	243-274	1697	1308	120-126
	F	91	130-161	258-298	1711	1391	125-147

4.2.4 Testing Environments

The test location for brackish water was at the Technical Implementation Unit for Brackish water Culture Karawang (-6.106192, 107.428710), at salinity around 20 ppt, and the location for freshwater/nucleus was at Research Institute for Fish Breeding (-6.371860, 107.623815). Both locations are in the West Java area close to the North Java Sea.

We stocked the tagged and mixed fingerlings from each batch of families in separate ponds on each site at an initial stocking density of ~5 fish per m^2. To minimize stress and mortality

during the stocking process, prior to stocking the brackish water ponds we temporarily reduced their salinity level from ~20ppt to 10ppt s by reducing the water level and re-filling the pond with freshwater from the irrigation waterway. During the grow out period, we fed the fish twice daily between 07:00 and 09:00 in the morning and between 15:00 and 17:00 in the afternoon with a commercial pellet diet containing 28% protein at a rate of 3-5% bodyweight. We also recorded water parameters such as dissolved oxygen, pH, temperature, and salinity daily using digital water quality tester.

4.2.5 Trait measurements

Following a grow-out period of 150 to 210 days, we harvested the fish, initially using three drags of a seine net, after which we drained the pond to catch all the remaining fish. We transferred all caught fish directly into a plastic container with diameter around 80 cm containing clove oil (~0.4 ml per litre of water) as an anaesthetic agent. This process was performed to avoid fish mortality due to handling stress during catching and measuring the phenotypic traits. The number of fish that survive at harvest ranged from 3 to 19 fish/family in brackish water pond and from 1 to 20 fish/family in freshwater pond. Survival rate per family was around 72.9±16.6% in brackish water ponds and 77.1±19.6% in freshwater ponds. During measurements, we weighed each fish for harvest weight (*HW*) using a digital scale to the nearest to 0.1 g. We also measured the standard length (*L*) with a ruler to the nearest 1 mm. From the individual stocking and harvest weights, we calculated daily growth coefficients (*DGC*, Bureau, D. et al., 2000) as:

$$DGC = \frac{HW^{\frac{1}{3}} - SW^{\frac{1}{3}}}{growing\ days}\ x\ 100 \qquad \text{Eq. [4.1]}$$

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

Similarly, we calculated individual growth rate for length, *GR(L)*, as:

$$GR(L) = \frac{SL_f - SL_0}{growing\ days} \qquad \text{Eq. [4.2]}$$

where SL_0 is standard length at stocking, SL_f is standard length at harvest, and growing days is the growing time between stocking and harvest.

We calculated the condition factor (*K*) according to Weatherley et al. (1987):

$$K = \frac{HW}{L^3} \times 10^5$$ Eq. [4.3]

With *HW* in grams and *L* in mm

For reproductive performance, we measured gonad weight and maturation stage for 6 fish per family in each environment. We measured gonad weight with digital scale (0.01g), and macroscopically determined the maturation score (*MS*) based on Legendre and Ecoutin (1989) with three stages for males and five stages for females. Gonadosomatic index (*GSI*) was determined as:

$$GSI = \frac{Gonad\ weight}{Body\ weight} \times 100$$ Eq. [4.4]

We analysed gonad weight and gonadosomatic index separately for each sex because the differences of the scores between sexes.

4.2.6 Data analysis

4.2.6.1 Descriptive statistics

We prepared and checked the raw data recorded in Microsoft Excel using R version 4.1 (R-Core-Team, 2021). In total, we obtained data for 1308 fish in brackish water and 1391 in freshwater ponds from 91 families (Table 4.1). We estimated descriptive statistics and checked for data anomalies using R version 4.1. We performed student t-test to evaluate whether *HW, L, DGC, GR (L), K*, and *GW* differed between environments.

4.2.6.2 Phenotypic and genetic parameters

We estimated genetic parameters for performance traits using a total of 2699 individual fish for *HW, L, DGC, GR (L)* and *K*. The animal model included fixed effects for pond, sex within pond and harvest age within pond for *HW* and *L*. Sex was coded as male (m) and female (f) and harvest age was calculated as the number of days between the stocking date and harvest date. We nested sex within ponds to take into account differences in age and sexual maturity of the different groups of families by estimating different effects of sex in each of the ponds.

We estimated the genetic correlations between environments were estimated with a bivariate animal model in ASReml version 4.1 (Gilmour, A R et al., 2015) that treats growth in different environments as different traits:

$$y_{ijkl} = \mu + \mathrm{POND}_i + \mathrm{SEX\ (POND)}_{i,j} + \mathrm{AGE\ (POND)}_{i,k} + \mathrm{INIT} + \mathrm{a}_l + \mathrm{e}_{ijkl}$$

Model [4.1]

where: $\mathbf{y_{ijkl}}$ is vector of single growth trait in fresh and brackish water; μ is overall mean; $\mathbf{POND_i}$ is fixed effect that accounts for both pond and batch effects (*i*=1– 3 for BW, and 4-6 for FW) ; $\mathbf{SEX\ (POND)_{i,j}}$ is the fixed effect of sex nested within pond (*j* =m, f); $\mathbf{AGE\ (POND)_{i,k}}$ is harvest age nested within pond; **INIT** is initial weight for *DGC*, initial length for GR and the ratio of initial weight and initial length for *K* which have been standardized by scaling it to a standard normal distribution; a_l is random additive genetic effect of the *l*-th individual; $\boldsymbol{e_{ijkl}}$ is random residual effect associated with an individual.

Common environmental effects (c^2) were expected in this study because families were reared separately from hatching jar into nursing hapas until tagging. However, solutions for c^2 could not be obtained because family effects are confounded with dam effects due to few half-sib families and the shallow pedigree information. We tried to fit the model with c^2 but the model was not converge. Without common environmental effect in the model, the solutions converged and the genetic correlations could be estimated. The full model was used to analyse the *DGC, GR (L)* and *K*. A simplified model that excluded the fixed effect for initial value (**INIT**) was used for *HW* and *L*.

We calculated the heritability as the ratio between additive genetic variance (σ_A^2) and phenotypic variance (σ_P^2), $\frac{\sigma_A^2}{\sigma_P^2}$. Genetic and phenotypic correlations between different traits in the same environment were also obtained from bivariate analysis. The animal effects were distributed as N(0,A⊗G) with the additive genetic variance covariance matrix (G) is $\begin{bmatrix} \sigma_{A,1}^2 & r_{A,12}\sigma_{A,1}\sigma_{A,2} \\ r_{A,12}\sigma_{A,1}\sigma_{A,2} & \sigma_{A,2}^2 \end{bmatrix}$ where $\sigma_{A,1}^2$ ($\sigma_{A,2}^2$) is the additive genetic variance of trait 1 (trait 2), and $r_{A,12}\sigma_{A,1}\sigma_{A,2}$ is the additive genetic covariance between trait 1 and trait 2. The residuals were distributed as N(0, I⊗R) with residual variance-covariance matrix (R) is $\begin{bmatrix} \sigma_{e,1}^2 & r_{e,12}\sigma_{e,1}\sigma_{e,2} \\ r_{e,12}\sigma_{e,1}\sigma_{e,2} & \sigma_{e,2}^2 \end{bmatrix}$ where $\sigma_{e,1}^2$ ($\sigma_{e,2}^2$) is the residual variance of trait 1 (trait 2), and $r_{e,12}\sigma_{e,1}\sigma_{e,2}$ is the residual covariance between trait 1 and trait 2. Genetic and phenotypic correlations among traits were calculated as the covariance divided by the product of the standard deviations of the two traits in the bivariate model.

For reproductive performance, we also estimated the genetic parameters with bivariate animal models that take into account fixed effects of pond and harvest age. For genetic analysis of gonad maturity, we reclassified the maturity score as mature (1) and immature (0) according to Legendre and Ecoutin (1989). We classified females as immature when they were in stage 1 to 3, and as mature when they were in stage 4 and 5. Whereas for males, they were classified as mature when they were in stage 2 to 3. Then we analysed males and females together with sex nested within pond as fixed effect.

We estimated the genetic correlation between the same traits measured on different (related) individuals in the brackish and freshwater ponds with the bivariate model above. For this model, the additive genetic variance-covariance matrix is $\begin{bmatrix} \sigma^2_{A,B} & r_{A,BF}\sigma_{A,B}\sigma_{A,F} \\ r_{A,BF}\sigma_{A,B}\sigma_{A,F} & \sigma^2_{A,F} \end{bmatrix}$ where $\sigma^2_{A,B}$ is the additive genetic variance for the traits in brackish water, $\sigma^2_{A,F}$ is the additive genetic variance for the traits in freshwater and $r_{A,BF}$ is the additive genetic correlation between brackish water and freshwater.

The covariances of residuals between environments was set to zero, as a fish performed in only one environment. The residual variance-covariance matrix is $\begin{bmatrix} \sigma^2_{e,B} & 0 \\ 0 & \sigma^2_{e,F} \end{bmatrix}$ where $\sigma^2_{e,B}$ is the residual variance for the trait in brackish water and $\sigma^2_{e,F}$ is the residual variance for the trait in freshwater.

4.3 Results

4.3.1 Descriptive statistics

The average salinity, morning and afternoon water temperature in the brackish water pond were 16.21 ppt, 29.57°C and 33.71°C, respectively. The salinity in the brackish water was highly variable, fluctuating over time between 6 and 25 ppt as shown in Figure 4.1. The lowest salinity was 6 ppt which occurred in raining period. The temperature profiles for the brackish and freshwater ponds are very similar (Figure 4.1).

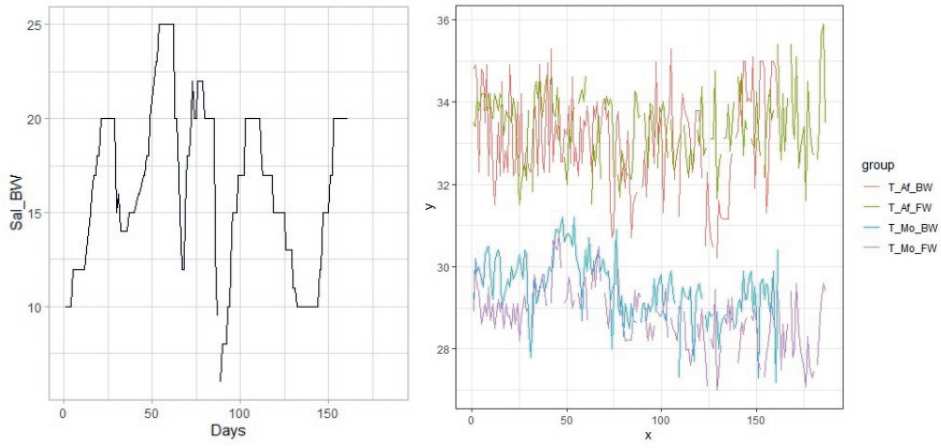

Figure 4.1 Daily salinity fluctuation in the brackish pond (left) and temperature (right) in the freshwater pond in the morning (T_Mo_FW) and afternoon (T_Af_FW), and in the brackish water pond in the morning (T_Mo_BW) and afternoon (T_Af_BW) during the experimental rearing period.

Grow out in brackish water pond resulted in 77.08% survival, and we recovered 1308 out of 1697 fish at harvest time after 120-126 days rearing period. In the freshwater pond, we observed higher survival of 81.82% or 1391 out of 1700 fish after 125-147 days rearing period. Descriptive statistics of *SW, HW, L, DGC, GR, K* and survival are shown in Table 4.2. The average stocking weight is similar between brackish water (16.11±7.79 g) and freshwater (15.65±7.75 g). *HW, L, DGC* and *GR* were higher for males compared to females in both brackish water and freshwater, but *K* was similar. However, the coefficient of variation for females was higher than males for all growth traits in both brackish water and freshwater. At harvest time, *HW* and *L* were higher in brackish water and significantly different ($P<0.05$) compared to freshwater. *DGC* in brackish water was higher (3.38±0.43) and significantly different ($P<0.05$) compared to freshwater (2.72±0.44). In brackish water, *GR(L)* during grow-out period was significantly higher compared to freshwater ($P<0.05$). The difference between K in brackish water (4.02±0.37) and in fresh water (3.98±0.36) was not significant. The regression coefficients and intercepts of log(*HW*) against log(*L*), were similar in brackish water and freshwater (Figure 4.2). Overall, brackish water leads to higher *HW, L, DGC, GR (L)* compared to freshwater.

Table 4.2 Number of observations (n), mean and coefficient of variation (CV in %) for stocking weight (*SW*) and harvest weight (*HW*), standard length (*L*), daily growth coefficient (*DGC*), growth rate (*GR (L)*), condition factor (*K*) and survival rate (*S*) of male and female *Oreochromis niloticus* in brackish water and freshwater

Trait	Brackish water					Freshwater				
	n	Male		Female		n	Male		Female	
		Mean	CV	Mean	CV		Mean	CV	Mean	CV
SW (g)	1697	16.8±7.49	44.58	16.1±8.60	53.27	1711	15.8± 7.28	46.07	15.1 ± 8.06	53.40
HW (g)	1308	324.7± 61.43*	18.92	255.0± 53.85*	21.14	1391	261.5 ±49.72*	19.01	201.7 ±46.60*	23.10
L (cm)	1308	19.9±1.32*	6.63	18.5±1.32*	7.12	1391	18.7±1.20*	6.45	17.1±1.30*	7.62
DGC ($g^{1/3}$ /day)	1308	3.5± 0.38*	11.02	3.12± 0.40*	12.42	1391	2.84±0.39*	13.81	2.5±0.45*	18.01
GR (L) (cm/day)	1308	0.10±0.011*	10.75	0.09±0.011*	11.74	1391	0.08±0.017*	21.68	0.07±0.012*	17.39
K	1308	3.95±0.38		4.05±0.36		1391	3.98±0.35		3.98±0.36	
S (%)		(77.07)					(81.29)			

*P< 0.05 Student-T test comparing brackish and freshwater ponds

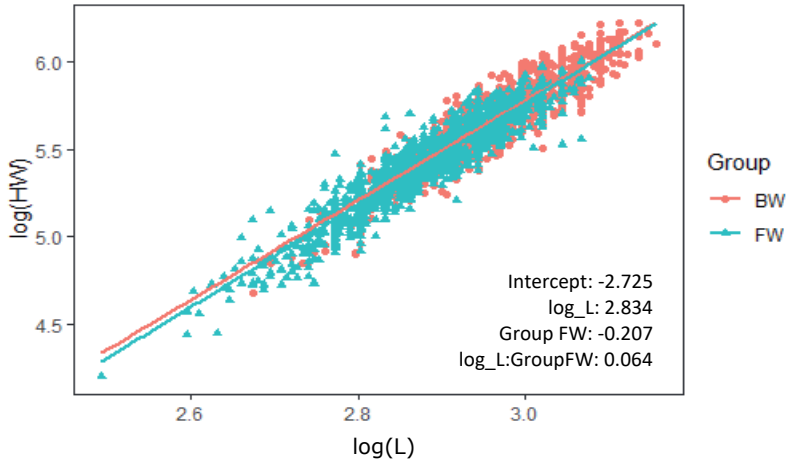

Figure 4.2 Log_{10}(*HW*) plotted against Log_{10}(*L*) for fish in brackish water (BW) and freshwater ponds (FW) (above) and the anova of analysis covariance between brackish and freshwater ponds (below). *HW*=harvest weight, *L*=length.

Table 4.3. Separate slopes from analysis of covariance for the relationship between length (*L*) and harvest weight (*HW*) brackish and freshwater ponds.

Anova	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Log_L	1	179.36	179.36	21903.095	2e-16
Group	1	0.20	0.20	24.108	9.66e-07
Interactions	1	0.02	0.02	2.129	0.145
Residuals	2686	22.00	0.01		

We evaluated the regression coefficient between log(*HW*) and log(*L*) for each group of fish in fresh and brackish water using a separate slopes analysis of covariance (Fig. 4.2). The estimated regression coefficients in brackish water and freshwater are 2.834 and 2.898, respectively, and are not significantly different (Interaction $P > 0.05$, Table 4.3). However, the intercept in brackish water was significantly higher than in freshwater (Group effect $P < 0.05$, Table 4.3).

Table 4.4 Means ($\bar{x}$), standard deviations (σ), coefficients of variation (CV in %) of gonad weight and GSI male and female from brackish water and freshwater

Traits	Brackish water				Freshwater			
	n	$\bar{x}$	σ	CV	n	$\bar{x}$	σ	CV
Gonad weight male (g)	299	1.03*	1.50	145.1	280	0.70*	0.76	109.7
Gonad weight female (g)	177	4.52*	3.56	78.6	198	2.42*	2.16	89.2
GSI male	299	0.31	0.50	159.7	280	0.28	0.31	111.1
GSI female	177	1.82*	1.40	77.2	198	1.26*	1.54	91.2

*p< 0.05 Student-T test comparing brackish and freshwater ponds

We evaluated the reproduction performance of males and females in both environments. Macroscopic analysis of gonad weight (Table 4.4) showed that gonad weight for both males and females and gonadosomatic index for females in brackish water was higher than in freshwater (P<0.05), but for males the difference in gonadosomatic index between brackish water and freshwater was not significant (P>0.05). Gonad maturity score varies between sexes and environment (Table 4.5).

Table 4.5 Gonad maturity score (MS) for males and females in brackish water and freshwater

Male			Female		
Stage	Brackish water	Freshwater	Stage	Brackish water	Freshwater
1	13 (4%)	8 (3%)	1	3 (2%)	2 (1%)
2	39 (13%)	57 (21%)	2	37 (20%)	47 (23%)
3	248 (83%)	212 (77%)	3	56 (30%)	67 (33%)
			4	72 (38%)	66 (33%)
			5	19 (10%)	20 (10%)

4.3.2 Genetic parameters of traits within environments

Genetic and phenotyping variances estimates for all traits in freshwater were lower compared to brackish water, except standard length (Table 4.6). The h^2 estimates for *HW, L, DGC, GR (L)* and *K* were moderate, from 0.35 to 0.50 with small standard error ranging from 0.06 – 0.09. All h^2 estimates for HW and L were higher in freshwater ponds compared to brackish water pond while for the ratio traits (*DGC, GR (L)* and *K*), h^2 estimates were higher

in the brackish water pond compared to the freshwater pond. We estimated the genetic parameters for reproduction traits separately for the sexes in both environment as shown in Table 4.7. The h^2 estimates varies from low to moderate (0.03-0.54). The genetic correlation of *GW* in both environments was higher in male (0.85) than in female (0.50). Genetic correlations for reproductive traits had higher standard errors than growth traits due to the smaller sample size (6 fish/family rather than ~ 20).

Table 4.6 Additive genetic variance (σ^2_A), total phenotypic variance (σ^2_P), heritability (h^2), genetic correlation (r_g), phenotypic correlation (r_p), and their standard errors (*se*) and genetic coefficient of variation (GCV) from bivariate analysis for harvest weight (*HW*), standard length (*L*), daily growth coefficient (*DGC*), growth rate (*GR (L)*) and condition factor (*K*) in brackish water and freshwater ponds

	Brackish water				Freshwater					
Trait	σ^2_A	σ^2_P	h^2 *(se)*	GCV[1)]	σ^2_A	σ^2_P	h^2 *(se)*	GCV[1)]	r_g	r_p
HW	1048.47	2958.8	0.35	10.2	790.57	2104.7	0.38	5.2	0.66	0.24
	(222.13)	(143.58)	(0.06)		(162.67)	(102.22)	(0.06)		(0.10)	(0.05)
L	0.48	1.24	0.39	3.2	0.53	1.30	0.41	2.7	0.73	0.29
	(0.09)	(0.06)	(0.07)		(0.10)	(0.06)	(0.07)		(0.09)	(0.06)
DGC	0.47×10^{-01}	0.11	0.43	6.5	0.37×10^{-01}	0.09	0.42	3.7	0.65	0.28
	(0.97×10^{-02})	(0.58×10^{-02})	(0.07)		(0.74×10^{-02})	(0.45×10^{-02})	(0.06)		(0.09)	(0.06)
GR (L)	0.30×10^{-04}	0.73×10^{-04}	0.42	4.5	0.26×10^{-04}	0.65×10^{-04}	0.39	2.8	0.74	0.30
	(0.84×10^{-05})	(0.47×10^{-05})	(0.09)		(0.67×10^{-05})	(0.39×10^{-05})	(0.08)		(0.31)	(0.09)
K	0.56×10^{-01}	0.11	0.50	5.3	0.54×10^{-01}	0.122	0.44	5.2	0.83	0.34
	(0.11×10^{-01})	(0.62×10^{-02})	(0.07)		(0.11×10^{-01})	(0.63×10^{-02})	(0.07)		(0.06)	(0.06)

[1)] GCV was calculated as: $\left(\frac{\sigma_A}{\mu}\right) * 100\%$

Table 4.7 Heritability (h^2) and genetic correlation (r_g) for gonad weight (*GW*), gonadosomatic index (*GSI*) and Maturity score (*MS*) in brackish and freshwater

Trait	Sexes	h^2 *(se)* Brackish water	h^2 *(se)* Freshwater	r_g *(se)*
GW	Male	0.13 (0.13)	0.38 (0.14)	0.85 (0.45)
	Female	0.30 (0.17)	0.21 (0.17)	0.50 (0.46)
GSI	Male	0.06 (0.11)	0.54 (0.14)	0.75 (0.74)
	Female	0.30 (0.16)	0.03 (0.15)	n.a.
MS	Both sexes	0.12 (0.07)	0.04(0.07)	0.47 (0.74)

se: standard error

The genetic correlations (r_g) between *HW, L, DGC,* and *GR(L)* within an environment (brackish or freshwater) were high (Supplement 4.1), varying from 0.81 to 0.99 and from 0.79 to 0.99, respectively. Similar trends appear in the phenotypic correlations (r_p). The estimated r_p were high in both environment from 0.86 to 0.99, except for r_p between *GR(L)* and *HW* in freshwater which was very high, and the software generated an estimated value >1 (Supplement 4.1). The r_p and r_g between *GR(L)* and *HW* in freshwater could not be estimated due to model convergence problems.

4.3.3 Genotype by environment interactions

The genetic correlations between brackish water and freshwater for *HW, L, DGC* and *GR (L)* were moderate ranging from 0.65 to 0.74. Figure 4.3 shows the patterns of the re-ranking of the parents of all families between brackish water and freshwater for *DGC* based on their estimated breeding values. The *DGC* interaction plot has many crossings and more families switch rank between environments resulting in lower genetic correlation than other traits. There are crossovers of high-ranking parents between the two environments, indicating that these families will perform differently in both environments. Genetic correlations for reproductive traits (*GW, GSI* and *MS*) show high variation between 0.47 and 0.85 with very high standard error (Table 4.7).

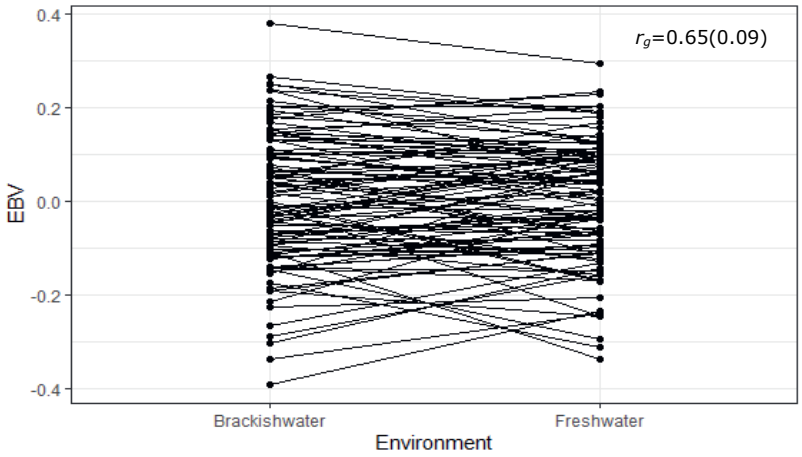

Figure 4.3. Reranking of parental estimated breeding valued (EBV) for daily growth coefficient (*DGC*) between brackish water and freshwater ponds. The genetic correlations (r_g) with standard error are included inside the plot.

4.4 Discussion

The objectives of our study were to investigate the extent of GxE interactions for growth and reproductive traits between brackish and freshwater ponds in the Indonesian Sukamandi tilapia strain being selected for improved salinity tolerance. This information will be important for redesigning and refining the breeding program. In the next three sections, we discuss the performance of the Sukamandi strain in brackish water, potential for further improvement of this strain and the implications for selective breeding program.

4.4.1 The Sukamandi strain performance in brackish water

The Sukamandi strain grew better in brackish water ponds than in freshwater ponds. At ~16.2 ppt *HW, DGC, L* and *GR (L)* were significantly higher in brackish water than in freshwater. Because physiological adaptation to elevated salinity requires energy to maintain osmotic homeostasis (Kültz, 2015) and the diverted energy to osmoregulation should reduce growth (Boeuf and Payan, 2001; Tseng and Hwang, 2008), we expected the opposite result based on previous studies of Nile tilapia, which has lower performance in brackish water than in freshwater ponds (Cnaani and Hulata, 2011; Fineman-Kalio, 2008; Kamal and Mair, 2005). The energetic requirements for osmoregulation to depend on the environment, and both hypo- and hyper-osmotic conditions require energy to maintain internal homeostasis. Consequently, the best growth performance of tilapia is achieved when they are in isosmotic conditions. Blue tilapia (*O. aureus*) and Mozambique tilapia (*O. mossambicus*) have higher

salinity tolerance than Nile tilapia and grow well in brackish water ponds up to 20 ppt for blue tilapia and close to full-strength seawater for Mozambique tilapia (Popma and Masser, 1999). Blue tilapia is in isosmotic conditions at salinities of 8 to 12 ppt based on blood chemistry (Semra et al., 2013). A molobicus hybrid tilapia strain has a salinity tolerance close to *O. mossambicus* (Mateo et al., 2004) and can reproduce in brackish water (Cnaani and Hulata, 2011). Our test condition were at salinity range around 16 ppt, which is closer to the isosmotic condition than freshwater. Our results indicate that the salinity tolerance of the Sukamandi strain (*Oreochromis* spp.) is closer to that of blue tilapia, than of Nile tilapia, most likely because this strain is a unique composite strain of Nile tilapia with introgressed salinity tolerance genes from blue tilapia (Yu et al., 2022). In addition, the strain has been selected for growth and survival in brackish water ponds for 4 generations, prior to the current experiment.

Our expectation was that fish would grow less in brackish water and have lower fecundity. However, we observed higher mean growth in brackish water, compared to freshwater, while gonadal development and maturation was comparable in both environments. The survival rate in brackish water (77 %) was close to survival rate in freshwater (81 %) which also indicates that the Sukamandi strain has a good salinity tolerance. The regression coefficient between *HW* and *L* in Figure 4.2 indicate whether fish grow thicker or thinner at the same length. When the slope below 3.0 indicates that fish become leaner and when the slope exceeds 3.0 indicates fish become fatter (Silva et al., 2015). In our study, the regression coefficients are 2.834 for brackish water and 2.898 for freshwater. They are statistically equal as indicated by the non-significant interaction effect ($P>0.05$) and close to 3, indicating that the fish were in a good condition in both environments. However, brackish water ponds provided a better environment for fish growth than freshwater ponds as indicated by the significant main effect of group ($P<0.05$). This suggests that salinity itself had no negative effects but that other aspects of the brackish water environment were biologically different and more beneficial for growth than the freshwater pond. A study by Dewi et al. (2012), found abundant phytoplankton and zooplankton, particularly *Calanus* sp and *Acartia* sp in brackish water ponds at 95,570 ind./L and 17,120 ind./L far higher than in freshwater ponds at RIFB at 604 ind/L for *Fillinia* sp. This additional natural food could boost fish growth and all related traits. Taken together our results show that the combination of inadvertent hybridization and mass selection have already enhanced the salinity tolerance of Sukamandi strain, making it a unique and valuable genetic resource for Indonesian tilapia breeding to produce superior strain for tilapia culture.

4.4.2 Potential for further improvement of the Sukamandi tilapia strain

The moderate heritabilities for all production-related traits indicate the presence of sufficient additive genetic variance for future selection on these traits to produce significant responses. Our estimate of h^2 for *HW* in the brackish water (0.35) is higher compared to what has been estimated for growth in intensive (0.19±0.07) and extensive systems (0.17±0.06) in molobicus hybrid tilapia strain (de Verdal et al., 2014) but is lower compared to what has been reported in previous studies for Nile tilapia grown in saline environments (0.53-0.57; Thoa et al. (2016) and Ninh et al. (2014)). It is possible that the large fluctuation in salinity in this study (6-25ppt) inhibited the Sukamandi strain from expressing its full genetic potential for growth. Alternatively, the difference in heritability could be due to strain differences. The Sukamandi strain is of hybrid origin and has been selected for 4 generations in brackish water.

We encountered problems with including common environmental effect (c^2) in our models, most likely due to shallow pedigree information and limited pedigree connections between families. Our dataset consisted mostly of full-sibs families and very few half-sib families. Consequently, genetic correlations between observed traits within and between environments were obtained from models without the common environmental effect and this can influence estimates of genetic variance. Maluwa et al. (2006a), Trọng, T. et al. (2013) and Omasaki et al. (2016) also reported that a multivariate model to estimate genetic correlation including a common environmental effect did not converge. Not including c^2 usually leads to over-estimated heritability's, as common environmental effects are absorbed in the additive genetic variance component. Expressing growth as *DGC* makes it less dependent of initial (i.e. pre-tagging) body weight which is the stage most affected by common environmental effects (Bureau, D. et al., 2000; Cho, 1992; Trọng, T. et al., 2013). This trait represents grow out period from stocking to harvest, while harvest weight is a cumulative growth from spawning to harvest. The estimated heritability for *DGC* in our study agrees with (Trọng, T. et al., 2013) who also omitted c^2 from the model.

Our estimates for all growth parameters showed substantial GxE between brackish and freshwater ponds. The between-environment genetic correlation for *DGC* was 0.65 (0.09), which suggests substantial re-ranking of genotypes between the two environments. Significant GxE was also reported for *HW* of Nile tilapia tested in brackish water and freshwater ponds by Luan et al. (2008b) at 0.45 ±0.09. The design of our experiment followed the recommendation of Sae-Lim et al. (2010) with ~1000 fish/environment with equal

representation of families, so we assume that our estimates are unbiased. However, the number of fish/environment did not solve the structure problem in our estimation when the number of half-sib families is low, resulted in not converge in the model. *GR(L), K, GSI, GW* and *MS* were also indicated substantial GxE between brackish and freshwater. However, small sample size at 6 fish per family for all reproductive traits due to logistical reason resulted in very high standard error, and not estimable GxE of GSI in females as shown in Table 7.

4.4.3 Implications for future breeding program

To date, the breeding program has been based on selection for own performance (mass selection) for harvest weight, conducted in various shrimp farm environments. The advantage of this breeding scheme is the high accuracy of selection due to selection on own growth performance in brackish water. However, this breeding scheme has several drawbacks related to high mortality of selection candidates during the grow out period in unpredictable salinity condition, security issues, and mortality during the transportation and adaptation from the testing site to the selection site in freshwater. There is also a potential risk in disease transfer from the test pond in brackish water to the brood stock facility in freshwater. Another issue is related to escapees during the grow-out of selection candidates that potentially spread into the natural brackish water environments. To avoid this, closed and restricted testing facilities should be implemented to prevent this threat. Furthermore, reducing the reproductive performance of tilapia in brackish water is desired and could be included in the selection criteria. However, this has positive impact to the environmental and negative consequences to breeding program. The negative consequence could be related to the mating problem to produce sufficient number of families in the next generation.

A shift from brackish water to freshwater pond for fish selection could minimize some of these downsides. However, when G×E interactions are strong, it could result in a reduction in genetic gain due to inaccurate selection of breeding candidates in freshwater (Mulder and Bijma, 2005). Re-ranking of genotypes is not substantial if the genetic correlation between environments is above 0.8 (Robertson, 1959). In this study, however, the genetic correlation was 0.65, which means that it is essential to incorporate information from full-sibs in brackish water. Further, combining own performance in freshwater with sib records in brackish water could increase the accuracy of selection and maximise the genetic gain. With own-performance records, we can exploit within-family variation to increase accuracy compared

to using only sib information. In practical terms, a sib selection program has several advantages: eliminating transportation costs of testing fish and selection candidate from brackish water to freshwater, and reducing chance of disease transfer from the test pond in BW to the nucleus in FW.

4.5 Conclusion

Our results show that brackish water ponds provided a positive environment for the Sukamandi strain. However, there was substantial re-ranking shown by genetic correlations of 0.65-0.74 in all observed growth traits. Based on this, we suggest to perform a nucleus breeding program in freshwater and incorporate sib information from brackish water ponds to increase the accuracy of breeding value estimation and to optimize genetic gain.

Acknowledgements

This study is part of the PhD project, funded by Koepon Foundation through grant number 4164016900, and Indonesia Endowment Fund for Education, Ministry of Finance Indonesia (LPDP - RI). This work was additionally funded through grant KB-35-001-001 under the Wageningen University & Research “Food Security and Valuing Water” program, which is supported by The Netherlands’ Ministry of Agriculture, Nature and Food Quality. We acknowledge the staff of Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries Indonesia and Technical Implementation Unit of Brackish Water Culture Karawang, Indonesia for providing the necessary help and facilities during the experiments.

Supplement 4.1 The genetic correlations (r_g) between *HW, L, DGC,* and *GR(L)* within an environment (brackish or freshwater)

Table 4.8 Estimated genetic correlations (above diagonal) and phenotypic correlations (below diagonal) between harvest weight (*HW*), standard length (*L*), daily growth coefficient (*DGC*), growth rate (*GR(L)*) and condition factor (*K*) in brackish water ponds. Standard errors are in brackets.

Trait	*HW*	*L*	*DGC*	*GR (L)*	*K*
HW	x	0.84 (0.04)	0.93 (0.02)	0.83 (0.04)	0.36 (0.12)
L	0.87 (0.01)	x	0.81 (0.05)	0.99 (0.00)	-0.15 (0.14)
DGC	0.97 (0.003)	0.87 (0.01)	x	0.89 (0.03)	0.35 (0.12)
GR (L)	0.87 (0.01)	0.99 (0.00)	0.89 (0.01)	x	-0.07 (0.14)
K	0.42 (0.03)	0.02 (0.04)	0.45 (0.03)	0.03 (0.04)	x

Table 4.9 Estimated genetic correlations (above diagonal) and phenotypic correlations (below diagonal). between harvest weight (*HW*), standard length (*L*), daily growth coefficient (*DGC*), growth rate (*GR(L)*) and condition factor (*K*) in freshwater ponds. Standard errors are in brackets.

Trait	*HW*	*L*	*DGC*	*GR(L)*	*K*
HW	x	0.87 (0.03)	0.95 (0.01)	Not estimable	0.25 (0.13)
L	0.88 (0.01)	x	0.79 (0.05)	0.99 (0.00)	-0.19 (0.14)
DGC	0.97 (0.00)	0.86 (0.01)	x	0.88 (0.03)	0.28 (0.13)
GR(L)	Not estimable	0.99 (0.00)	0.89 (0.01)	x	-0.03 (0.20)
K	0.34 (0.03)	-0.06 (0.04)	0.37 (0.03)	0.02 (0.07)	x

Chapter 5

Genetic parameters and genotype by environment interaction of log transformed variance (LnVar) in growth of a salinity tolerant strain of tilapia ("*Sukamandi*") grown in co-culture with shrimp

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Abstract

The culture of tilapia in brackish water, alone or in combination with shrimp, can provide an opportunity to establish a profitable and sustainable aquaculture system for small-scale farmers in Indonesia. To increase production in brackish water environments, fish farmers need resilient tilapia capable of consistent and predictable growth performance. Previously, we showed that temporal deviations from expected individual growth trajectories, expressed as log-transformed variance of growth deviations (*LnVar*), is heritable and can be used to describe variation in growth over time. The objectives of this study were to estimate genetic parameters for growth and for *LnVar* in the Sukamandi strain of tilapia, in brackish water. We produced 102 tilapia families and randomly assigned fingerlings to grow-out in co-culture with shrimps or to grow-out in monoculture. We recorded weight at five time points during grow-out, but due to mass mortality between t_4 and t_5, we could only use weight records at 4 time points ($W_1 - W_4$) to calculate *DGC* and *LnVar*. The heritability (h^2) estimates of *LnVar* were 0.12 in co-culture and zero in monoculture. Genetic correlations between co-culture and monoculture were high for W_4 and *DGC* (0.96-0.99) and moderate for *LnVar* (0.67). Genetic correlations of *LnVar* with *DGC* and W_4 using pooled data from co-culture and monoculture were positive and moderate (0.62 ± 0.12 and 0.40 ± 0.14, respectively), suggesting that selection for growth will increase variation in growth. Surprisingly, *LnVar* was more heritable in co-culture than in monoculture, and we found moderate GxE between co-culture and monoculture. This suggests that genetic variation for growth consistency is expressed in the presence of shrimp. To enhance predictable fish growth in the brackish water environment, we recommend that fish breeding programs collect repeated records on body weight and include both *LnVar* and growth in the breeding goal, with appropriate economic weights to maximize profit.

Keywords: Variance of deviation of individual growth, brackish water, co-culture, heritability, genotype by environment interaction

5.1 Introduction

The culture of tilapia in brackish water, alone or in combination with shrimp, can provide an opportunity to establish a profitable and sustainable aquaculture system for small-scale farmers in Indonesia. Following severe disease outbreaks that have caused repeated failures in shrimp farming, several adaptation strategies have been applied by small-scale farmers including shifting to other species, especially tilapia, using rotational cropping and co-culture of shrimp with tilapia (Setyawan et al 2022; Modadugu and Acosta, 2004; Fitzsimmons and Shahkar, 2017; Martínez-Porchas et al., 2010). Several studies indicate that the addition of tilapia to shrimp ponds can improve feed efficiency, reduce the incidence and severity of bacterial and viral infections in shrimp, and provide additional income for fish farmers (reviewed by Martínez-Porchas et al., 2010; Fitzsimmons and Shahkar, 2017). Saline tolerant tilapia is suitable for co-culture with shrimp in brackish water ponds because it is able to utilize different niches than shrimp and can tolerate the same salinity range between 1-30 ppt (Ray and Lotz, 2017, Jaffer et al., 2020).

In brackish water ponds, the level and fluctuations of salinity are important abiotic factors influencing fish growth (Boyd, 2017; Cui and Chui, 2017; Ariadi et al., 2023). To cope with these salinity fluctuations, fish allocate energy to maintain osmotic homeostasis, which limits the energy available for growth (Kültz, 2015; Boyd, 2017; Bal et al., 2021). Furthermore, most small-scale farms with extensive and semi-intensive production systems grow tilapia in non-aerated ponds. The use of aeration is not feasible for small-scale farmers with low-income status (Setyawan et al., 2022b; Martínez-Porchas et al., 2010). Without aeration, ponds show daily diurnal dissolved oxygen (DO) fluctuations, which creates a challenging environment (Mengistu et al., 2020a). DO is one of the main limiting factors that affect fish productivity, particularly in determining food intake, growth, and efficient metabolic processes (Mengistu et al., 2020b, Brauner and Richards, 2020). Furthermore, the addition of shrimp in direct co-culture could introduce stressors to tilapia due to social factors, such as increased density, which may become a limiting factor as oxygen consumption increases with biomass (Boyd, 2017; Milstein and Hernández, 2017).

Fish farmers need resilient tilapia capable of consistent and predictable growth performance to increase production in brackish water environments. Growth consistency can be measured using the variance of deviations between observed and expected performance from longitudinal records on body weight, known as *LnVar*. Studies by Mengistu et al. (2022) and Aththar et al. (Chapter 2) showed that *LnVar* for growth of Nile tilapia in ponds with daily

diurnal DO fluctuations is heritable and can be used to describe variation in growth over time. Therefore, *LnVar* could be improved through selective breeding.

In this study, we used growth data from the tilapia breeding program at the Research Institute for Fish Breeding (RIFB), Indonesia. The RIFB has been conducting a small-scale breeding program for five generations, focusing on improving the growth performance of tilapia in brackish water using the Sukamandi tilapia, a unique strain composed of Nile tilapia (*Oreochromis niloticus*) and blue tilapia (*Oreochromis aureus*) (Setyawan et al., 2022b; Yu et al., 2022). Our previous result showed that Sukamandi tilapia grew better in brackish water ponds than in freshwater ponds (Setyawan et al., 2022a). Furthermore, a study by Setyawan et al. (2022b) indicates that co-culturing tilapia and shrimp is the preferred culture system for most smallholder farmers in coastal area of Java Island in Indonesia. The Sukamandi tilapia is selected under monoculture conditions. Due to genotype by environment interaction (GxE), genotypes may perform differently in different environments (Falconer and Mackay, 1996), meaning that the performance of the Sukamandi tilapia in monoculture may not predict its performance in co-culture. In this context, the objectives of this study were: 1) to estimate genetic parameters in Sukamandi tilapia for growth and consistency of growth, measured with *LnVar*, in both brackish water co-culture and monoculture; 2) to estimate genetic correlations of growth and *LnVar* between co-culture and monoculture; and 3) to estimate the genetic correlation of growth with *LnVar*.

5.2 Materials and Methods

5.2.1 Family production and nursery

The fish used in this experiment were produced from generation 5 of the Sukamandi tilapia at the Research Institute for Fish Breeding, Indonesia. We produced 102 families from 39 sires and 82 dams over a period of 21 days in breeding cages measuring 4 m^2 each (2 × 2m), suspended in three 200 m^2 earthen ponds. Each of these cages was stocked with one male and three females, with males introduced to the cages one day before the females. After 7 days of mating, fertilized egg or larvae were checked and collected from a female's mouth. In total, there were 3 collection dates for egg and larvae with one week intervals (Figure 5.1). After collection, eggs were incubated in hatching jars until yolk-sac absorption. We transferred swim-up fry from each family into separate nursery cages within the earthen pond. For this, we randomly sub-sampled 200 fry and stocked them at a density for nursing of 50 fish per m^2. The nursery period ranged from 84-98 days. At the end of this period, we randomly selected, weighed, and tagged 40 individuals from each family using PIT (Passive

Integrated Transponder) tags, recording their identification number and stocking weight (W_1). For each family, 20 fingerlings were randomly assigned to co-culture treatment with shrimp and similarly 20 fish were randomly assigned to monoculture. After tagging, the fish were transferred into cages for conditioning with a minimum feeding rate of 2% body weight before transportation to the brackish water ponds.

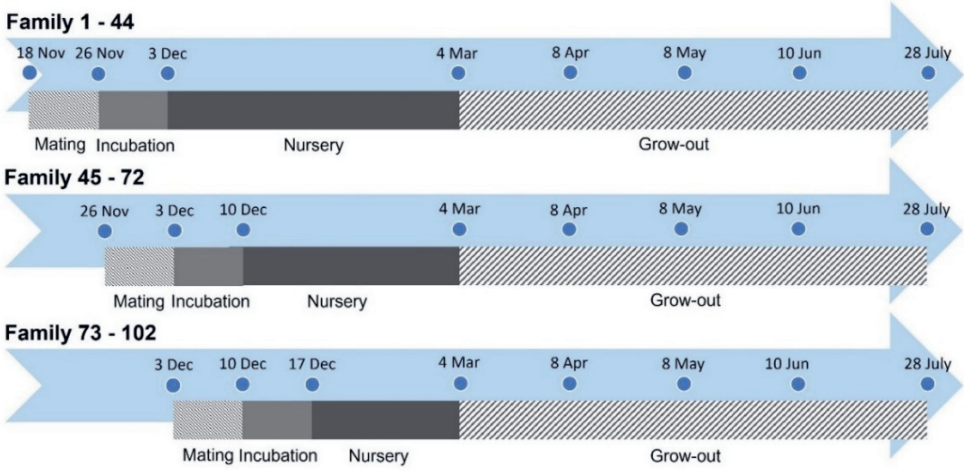

Figure 5.1 Schematic overview of the experimental design showing different lifecycle phases of the three family cohorts Sukamandi tilapia.

5.2.2 Testing Environments

Brackish water ponds were located at the Technical Implementation Unit for Brackish water Culture (TIUB) Karawang (-6.106192, 107.428710). We utilized two brackish water ponds measuring 25 x 50 m^2 each and installed 27 cages sized at 3 x 5 x 1 m^2 per pond. In pond 1, we assigned 14 cages for co-culture and 13 cages for monoculture treatments, while in pond 2, we assigned 13 cages for co-culture and 14 cages for monoculture treatments (Supplement 5.1). From the 20 fish per family, we distributed 5 fish into each of 4 cages for one treatment. Per cage we stocked 15 families, resulting in an initial stocking density of 5 fish per m^2. To minimize stress and mortality during the stocking process, we temporarily reduced the salinity level before stocking from approximately 20 ppt to 10 ppt by lowering the water level and replenishing the pond with fresh water from the irrigation waterway. For the co-culture treatment, Whiteleg shrimp (*Penaeus vannamei*) weighing 5 grams were stocked one week before the fish. During the grow out period, we fed the fish twice daily with a commercial pellet diet containing 28% protein, at a daily rate of 3% of their bodyweight. Additionally, we conducted daily monitoring of water parameters such as DO,

pH, temperature, and salinity, using a digital water quality tester. Daily average rainfall information for the experimental location was obtained from Climate SERV (ClimateSERV, 2024). To acquire this data, we set the brackish water ponds location in TIUB as the area of interest and selected the average time-series observations from the UCSB CHIRP Rainfall data source to show daily average rainfall from 9 March – 2 July 2021. This period was chosen because it corresponds with the timeframe for daily monitoring of water quality parameters in ponds.

5.2.3 Trait measurements

Before each measurement, we anaesthetized fish with clove oil to minimize handling stress. For each fish, we measured the weights at 5-time points (W_1 – W_5): stocking time (t_1: day 1), 3 interval time points ($t_{2\text{-}4}$: 35, 65 and 98 days) and harvest time (t_5: 146 days). Fish from pond 1 and 2 were always measured on two consecutive days. We observed mass mortality between t_4 and t_5 in pond 2 during the grow-out period (Table 5.1). Consequently, we used longitudinal measurements of weight at four time points (W_1 – W_4). The four weight measurements were used to calculate log transformed variance (*LnVar*) and daily growth coefficient (*DGC*). In this study, we calculate *LnVar* based on individual expected growth trajectories (Aththar et al., Chapter 2). *LnVar* is calculated from the deviations of observed weights from the expected weights of the individual at timepoints W_1 to W_4.

We calculated the expected weight of individual fish at age *d* as:

$$W_{exp,id}^{\frac{1}{f}} = a + DGC_i * d \qquad \text{Eq. [5.1]}$$

where $W_{exp,id}$ is the expected weight of fish *i* at age *d, f* is the overall weight exponent, DGC_i is daily growth coefficient of fish *i* and *d* is fish age. To estimate *f*, we fitted an exponential curve to the observed weights of all the fish:

$$W_{obs,id} = a + b_i * d^f \qquad \text{Eq. [5.2]}$$

Where $W_{obs,\,id}$ is the weight of fish *i* at age *d*, *a* is the intercept, b_i is the slope of the non-linear regression for fish *i*, *d* is the fish age and *f* is the overall weight exponent. The weight exponent *f* was estimated using the *nls* function in R (RStudio-Team, 2022). Then, to calculate *DGC* for fish *i* (DGC_i) in Eq. [5.1], we transformed the 4 observed weights per fish as $W_{obs,id}^{\frac{1}{f}}$ to linearize the growth curve and we estimate DGC_i as the slope of the linear regression of $W^{\frac{1}{f}}$ on the age of the fish *i* at 4 time points.

Per fish, we then calculate the deviations (dev_{it}) as:

$$dev_{it} = W_{obs,it}^{\frac{1}{\bar{f}}} - W_{exp,it}^{\frac{1}{\bar{f}}} \quad \text{Eq. [5.3]}$$

Where dev_{it} is the deviation of observed weight from expected weight of fish *i* at time point *t*, $W_{obs,it}$ is the observed body weight of fish *i* at time point *t* and $W_{exp,it}$ is the expected body weight of fish *i* at time point *t*. Next, for each fish, we calculate the variance of the resulting 4 deviations (*Var-dev*). Finally, we log-transformed *Var-dev* using the natural logarithm to obtain log-transformed variance (*LnVar*), which is the commonly used scale to express genetic variation in residual variance (Hill and Mulder, 2010).

5.2.4 Phenotypic and genetic parameters

Data preparations, descriptive statistics and data checks were conducted using R software version 4.0.2 (R-Development-Core-Team, 2022) running on RStudio version 1.3.959 (RStudio-Team, 2022). Phenotypic and genetic variances of *LnVar*, W_4 and *DGC* were estimated using ASReml version 4.2 (Gilmour, A. R. et al., 2015) fitting an animal model with a pedigree relationship matrix. We used the following animal model:

$$y_{ijklm} = \mu + \mathrm{Age1}_i + \mathrm{Sex\ (Pond)}_j + \mathrm{Cage\ (Pond)}_k + \mathrm{a}_l + \mathrm{Family}_m + \mathrm{e}_{ijklm}$$

Model [5.1]

where: $\mathbf{y_{ijklm}}$ is the vector of one of the traits *LnVar*, W_4 and *DGC* ; μ is overall mean; $\mathbf{Age1_i}$ is fixed effect of initial age (*i* = 84, 91, 98 days) as the indicator for the difference of production batch and initial weight; $\mathbf{Sex\ (Pond)_j}$ is fixed effect of sex nested within pond (*j*=m, f indicates male and female within each of Pond 1 and Pond 2); $\mathbf{Cage(Pond)_k}$ is fixed effect of cages nested within pond (*k* = Cage 1 - 27 for Pond 1 and Cage 28 – 54 for Pond 2); a_l is random additive genetic effect of the *l*-th individual; $\mathbf{Family_m}$ is random effect due to common environmental effects for individuals in family *m* (*m* = 1 - 102); and $\mathbf{e_{ijklm}}$ is random residual effect associated with an individual. The model included **Family** as random common environmental effect (c^2), which reflected the separate rearing of full-sib groups in family-specific hatching jars and nursing hapas until tagging (91-105 days) before the direct transfer to the grow-out ponds. We calculated heritability (h^2) as the ratio between additive genetic variance (σ_A^2) and phenotypic variance (σ_P^2), $\frac{\sigma_A^2}{\sigma_P^2}$. Common environmental effect (c^2) was

calculated as the ratio between common environmental variance (σ_c^2) and phenotypic variance (σ_P^2), $\frac{\sigma_c^2}{\sigma_P^2}$.

We estimated the genetic correlations between the same traits measured on different (related) individuals in co-culture and monoculture treatments with a bivariate model equal to Model [5.1], but excluding c^2. The estimates of genetic correlations with including c^2 did not converge. Without c^2 in the model, the solutions converged and the genetic correlations could be estimated. The animal effects for bivariate model were distributed as N(0,G⊗C) where the additive genetic variance covariance matrix (C) is $\begin{bmatrix} \sigma_{A,M}^2 & r_{A,MC}\sigma_{A,M}\,\sigma_{A,C} \\ r_{A,MC}\sigma_{A,M}\,\sigma_{A,C} & \sigma_{A,C}^2 \end{bmatrix}$ and G is the pedigree relationship matrix, $\sigma_{A,M}^2$ and $\sigma_{A,C}^2$ is the additive genetic variance for the traits in monoculture and co-culture. $r_{A,MC}\sigma_{A,M}\,\sigma_{A,C}$ is additive genetic covariance between monoculture and co-culture. The covariance of residuals between treatments was set to zero, as a fish performed in only one treatment. The residual variance-covariance matrix is $\begin{bmatrix} \sigma_{e,M}^2 & 0 \\ 0 & \sigma_{e,C}^2 \end{bmatrix}$ where $\sigma_{e,M}^2$ is the residual variance for the trait in monoculture and $\sigma_{e,C}^2$ is the residual variance for the trait in co-culture.

To estimate genetic and residual correlations between the different traits *LnVar*, W_4 and *DGC* measured in the same animal, we used combined data from both co-culture and monoculture treatments. We estimated genetic correlations between the traits with a bivariate model equal to model 1, but excluding c^2. The estimates of genetic correlations between *LnVar* and W_4 and *DGC* while including c^2 did not converge. However, without c^2 in the model, the solutions converged and the genetic correlations could be estimated. The animal effects for the bivariate model were distributed as N(0,G⊗C) where the additive genetic variance covariance matrix (C) is $\begin{bmatrix} \sigma_{A,1}^2 & r_{A,12}\sigma_{A,1}\sigma_{A,2} \\ r_{A,12}\sigma_{A,1}\sigma_{A,2} & \sigma_{A,2}^2 \end{bmatrix}$, and G is the pedigree relationship matrix, $\sigma_{A,1}^2$ and $\sigma_{A,2}^2$ are the additive genetic variances of trait 1 and trait 2, and $r_{A,12}\sigma_{A,1}\sigma_{A,2}$ is the additive genetic covariance between trait 1 and trait 2. The residuals were distributed as N(0, I⊗R) where the residual variance-covariance matrix (R) is $\begin{bmatrix} \sigma_{e,1}^2 & r_{e,12}\sigma_{e,1}\sigma_{e,2} \\ r_{e,12}\sigma_{e,1}\sigma_{e,2} & \sigma_{e,2}^2 \end{bmatrix}$, and I is an identity matrix, $\sigma_{e,1}^2$ and $\sigma_{e,2}^2$ are the residual variances of trait 1 and trait 2, and $r_{e,12}\sigma_{e,1}\sigma_{e,2}$ is the residual covariance between trait 1 and trait 2. Genetic and phenotypic correlations between traits were calculated as the covariance divided by the product of the standard deviations of the two traits.

5.3 Results

5.3.1 Descriptive statistics

5.3.1.1 Environmental parameters

The salinity fluctuated over time between 7 - 22 ppt in Pond 1 and 7 – 23 ppt in Pond 2 (Figure 5.2). The missing data points in Figure 5.2 were due to high rain intensity when it was not possible to conduct measurements. The temperature profiles for Pond 1 and 2 were similar, averaging approximately 28.6°C in the morning and 33.4°C in the afternoon. DO and pH were measured once per day in the morning during the grow out period. DO fluctuated between 2.4 – 6.3 ppt in Pond 1 and 1.9 – 6.3 ppt in Pond 2. pH levels were comparable in both Ponds 1 and 2, with values around 7.7. According to field observations and information from ClimateSERV (2024), there was a significant increase in rainfall volume between time points 4 and 5 (Figure 5.2), which led to the flooding in Pond 2.

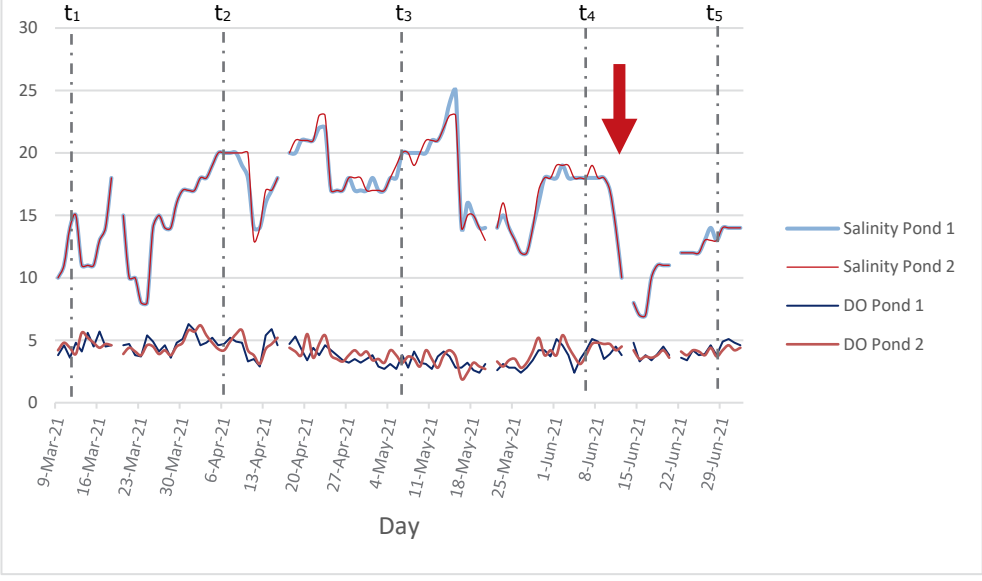

Figure 5.2 Average daily salinity (ppt) and dissolved oxygen (DO) (mg/l) in brackish water ponds 1 and 2 during the grow-out period of Sukamandi tilapia. Vertical dash-dot lines indicate the time points (t_{1-5}) for phenotype data collection. Missing lines represent days with no observations for salinity and DO. The red arrow highlights an increase in rainfall.

5.3.1.2 Survival rate

We initially stocked 4034 fish. Table 5.1 shows the number of surviving fish at each time point. Survival rates in pond 1 decreased from 66.2% and 69.5% in t_4 to 55.0% and 53.0% in t_5 for co-culture and monoculture treatments, respectively. In pond 2, mass mortality occurred between t_4 and t_5 due to flooding, reducing survival rates from 84.0% and 83.5% at t_4 to 0.7% and 0.1% at t_5 for the co-culture and monoculture treatments, respectively.

Table 5.1 Total number and survival rate of Sukamandi tilapia at time points 1 – 5 in ponds 1 and 2 with co-culture and monoculture treatments

Ponds	Treatments		Time points				
			1	2	3	4	5
1	Co-culture	N	1051	908	861	696	578
		%		86	82	66	55
	Monoculture	N	971	834	803	675	515
		%		86	83	70	53
2	Co-culture	N	965	828	813	811	7
		%		86	84	84	1
	Monoculture	N	1047	888	881	874	1
		%		85	84	83	0

5.3.1.3 Descriptive statistics of observed traits

Descriptive statistics are shown in Table 5.2. Overall, there were no significance differences between co-culture and monoculture for W_{1-5}, *DGC* and *LnVar*. The overall weight exponent(*f*) for Sukamandi tilapia in this experiment was estimated to be 2.34 and was used to calculate *DGC*.

Table 5.2 Mean and standard deviation (*sd*) for weights (W_{1-5}; in gram), daily growth coefficient (*DGC*) ($g^{1/2.34}$/day), and log transformed variance of growth deviations (*LnVar*) of Sukamandi tilapia in co-culture and monoculture treatments

	Co-culture			Monoculture		
	mean (sd)	min	max	*mean (sd)*	min	max
W_1	10.9 (3.0)	4.8	23.9	10.7 (3.2)	4.5	28.2
W_2	42.5 (9.8)	8.00	74.0	41.5 (10.1)	12.0	77.0

W_3	79.8 (19.0)	24.0	145.0	79.0 (19.5)	26.0	159.0
W_4	133.1 (31.0)	31.0	242.0	129.4 (31.2)	27.0	262.0
W_5	194.0 (44.9)	86.3	324.3	194.5 (47.5)	83.0	358.0
DGC	0.05 (0.01)	0.016	0.076	0.05 (0.01)	0.023	0.082
LnVar	-3.31 (1.36)	-11.38	-0.23	-3.32 (1.35)	-12.47	-0.33

5.3.2 Genetic analysis of observed traits

5.3.2.1 Estimated fixed effects on all observed traits

The fixed effects of **Sex (Pond)** and **Cage (Pond)** were significant (P<0.05) for all traits. Although, the fixed effect of **Age1** was not significant for *DGC* and *LnVar*, it was kept in the model due to its biological reason. Table 5.3 shows the estimated effects of Sex within Pond on W_4, *DGC* and *LnVar*.

Table 5.3 Estimated effects of **Sex (Pond)** on W_4, *DGC* and *LnVar*

Traits	Sex	Pond 1	Pond 2
W_4	Male	0.00	0.00
	Female	-24.80	-29.70
DGC	Male	0.00	0.00
	Female	-0.50×10^{-02}	-0.60×10^{-02}
LnVar	Male	0.00	0.00
	Female	-0.24	0.09

5.3.2.2 Genetic parameters of observed traits

The heritability estimate for *LnVar* in co-culture was 0.12, while in monoculture, it was 0.00 (Table 5.4). In co-culture, the heritabilities for W_4 and *DGC* were 0.09 and 0.11, respectively, while in monoculture, the estimates were 0.04 for W_4 and 0.11 for *DGC* . Common environmental effect estimates were found for W_4, *DGC* and *LnVar* in both co-culture and monoculture treatments, ranging from 0.02 – 0.13.

Table 5.4 Genetic variances (σ^2_A), phenotypic variances (σ^2_P), heritability (h^2) and common environmental effect (c^2) of log transformed variance of growth deviations (*LnVar*), daily growth coefficient (*DGC*) and weight (W_4) and their standard errors (*se*) of Sukamandi tilapia in brackish water co-culture and monoculture

Trait	Co-culture				Monoculture			
	σ^2_A	σ^2_P	h^2	c^2	σ^2_A	σ^2_P	h^2	c^2
LnVar	0.21 (0.12)	1.67 (0.70×10^{-01})	0.12 (0.07)	0.02 (0.03)	0.12×10^{-06} (0.00)	1.74 (0.68×10^{-01})	0.00 (0.00)	0.09 (0.02)
W_4	57.47 (45.61)	637.71 (27.11)	0.09 (0.07)	0.08 (0.03)	29.63 (47.03)	770.04 (30.80)	0.04 (0.06)	0.08 (0.03)
DGC	0.56×10^{-05} (0.47×10^{-05})	0.52×10^{-04} (0.25×10^{-05})	0.11 (0.09)	0.13 (0.04)	0.65×10^{-05} (0.54×10^{-05})	0.58×10^{-04} (0.27×10^{-05})	0.11 (0.09)	0.11 (0.04)

5.3.2.3 Genetic correlations between co-culture and monoculture treatments

Table 5.5 shows the genetic correlation (r_g) of *LnVar*, W_4 and *DGC* between co-culture and monoculture treatments. The genetic correlations (r_g) between co-culture and monoculture were high for growth traits, with 0.96 for W_4 and 0.99 for *DGC* , while the r_g for *LnVar* was moderate at 0.67.

Table 5.5. Genetic correlations (r_g) of weight (W_4), daily growth coefficient (*DGC*) and log transformed variance of growth deviations (*LnVar*), and and their standard errors (*se*) of Sukamandi tilapia between co-culture and monoculture treatments. Heritability (h^2) estimates from the bivariate models that omit c^2 are included.

Trait	r_g	h^2	
		Co-culture	Monoculture
W_4	0.96 (0.05)	0.34 (0.07)	0.29 (0.06)
DGC	0.99 (0.01)	0.52 (0.08)	0.45 (0.08)
LnVar	0.67 (0.16)	0.28 (0.10)	0.09 (0.07)

5.3.2.4 Genetic correlations between traits

Table 5.6 shows the genetic correlations between traits *LnVar*, W_4 and *DGC* , estimated using combined data from co-culture and monoculture treatments. The genetic correlations of *LnVar* with W_4 and *DGC* were positive and moderate (0.62 ± 0.12 and 0.40 ± 0.14, respectively). A high genetic correlation was observed between W_4 and *DGC* , with an estimate of 0.87 ± 0.05.

Table 5.6. Estimated genetic correlations (above diagonal) and phenotypic correlations (below diagonal) of log transformed variance of growth deviations (*LnVar*), weight (W_4) and daily growth coefficient (*DGC*) and their standard errors (*se*) for Sukamandi tilapia from the combined data of co-culture and monoculture treatments in brackish water ponds.

	LnVar	W_4	*DGC*
LnVar	X	0.62 (0.12)	0.40 (0.14)
W_4	-0.04 (0.03)	X	0.87 (0.05)
DGC	-0.09 (0.03)	0.88 (0.01)	X

5.4 Discussion

5.4.1 Growth performance of Sukamandi tilapia in brackish water co-culture with shrimp

We found no significant differences in Sukamandi tilapia growth between co-culture and monoculture, indicating that the addition of shrimp in co-culture did not affect tilapia growth (Perschbacher, 2017; Milstein and Hernández, 2017). The production performance of species grown in direct co-culture within brackish water ponds is influenced by the tolerated range of salinity for both species (Fitzsimmons and Shahkar, 2017; Martínez-Porchas et al., 2010; (Ray and Lotz, 2017; Jaffer et al., 2020). Earlier results showed that Sukamandi tilapia thrives within the tolerated salinity range of 1-30 ppt for Whiteleg shrimp (Chapter 3), further highlighting its potential for co-culture within brackish water environments.

The estimated growth exponent (*f*) of 2.34 indicates that the Sukamandi tilapia used in this study showed more linear growth compared to GIFT tilapia, which have a growth exponent of 1.77 (Aththar et al., Chapter 2). To compare the growth patterns of Sukamandi tilapia and GIFT tilapia, we plotted the growth curves using the growth exponents and the average *DGC* estimated in this chapter and in Chapter 2 for GIFT tilapia in non-aerated ponds. The results show that the GIFT tilapia have a steeper growth curve than the Sukamandi tilapia, and that GIFT tilapia grew faster than Sukamandi tilapia (Supplement 5.2). A direct comparison of GIFT and Sukamandi tilapia is not available, but growth was measured in both studies without aeration and at a comparable average temperature (27.3°C for GIFT tilapia and 28.6°C for Sukamandi tilapia) and feeding rate at 3% of body weight per day. The combination of salinity and DO fluctuations may have added pressure on the growth performance of Sukamandi tilapia compared to the growth of GIFT in Chapter 2 that did not experience salinity fluctuations. Additionally, GIFT tilapia has already undergone selection for growth over a longer period of 17-18 generations (Mengistu et al., 2022) than Sukamandi tilapia that have been selected for growth for five generations (Setyawan et al., 2022a). This difference in the number of generations of selection is a likely contributor to the difference in growth performance.

5.4.2 Genetic parameters and implications for breeding programs

The heritability estimate of *LnVar* is higher in co-culture (0.12) than in monoculture (0.00). These heritability estimates were lower than the previous estimate for *LnVar* in GIFT tilapia grown in non-aerated ponds (0.28) (Aththar et al., Chapter 2). The low heritability of *LnVar* observed in this experiment may be due to common environmental effects, resulting from common family rearing until tagging for 91 - 105 days. The estimate in GIFT tilapia was obtained with mass produced fry that were nursed together in the same hapa until tagging, such that common environmental effects were absent (Mengistu et al., 2020a). Further, the heritability estimates for weight at timepoint 4 in co-culture and monoculture (0.09 and 0.04, respectively) were also lower compared to the estimate from the previous generation of the Sukamandi tilapia (h^2 = 0.35; Setyawan et al., 2022a). We also observed significantc^2 estimates for weight in both co-culture and monoculture. In the previous study by Setyawan et al. (2022a), heritability was estimated using a model that excluded c^2 due to issues with model convergence. In this study, omitting c^2 from the model to estimates h^2 also results in a higher h^2 (Table 5.5). Excluding c^2 typically results in overestimated heritabilities when common environmental effects accounts for a significant proportion of phenotypic variance (Gjerde et al., 2012; Nguyen et al., 2017). In tilapia selective breeding, several factors can introduce common environmental effects, such as prolonged periods of separate family rearing (Maluwa et al., 2006b; Thodesen et al., 2011; Gjerde et al., 2012; Trọng, T.Q. et al., 2013; Nguyen et al., 2017) and differing environmental conditions during early life stages, including maternal effects via egg size and initial mouth brooding (Jonsson and Jonsson, 2014; Khaw et al., 2009). A study in tilapia by (Rutten et al., 2005) has shown that the c^2 effect for weight in Nile tilapia diminishes over time. However , the current c^2 value for W_4 suggests that the grow out period in this study was too short for the common environmental effect to disappear.

Surprisingly, *LnVar* showed a higher heritability estimate in co-culture than in monoculture (although not significantly different from 0). A higher heritability in co-culture would suggests that there is genetic variation for variability of growth of "Sukamandi" tilapia that comes to expression in the presence of shrimp. The presence of GxE for *LnVar* between co-culture and monoculture also indicates that *LnVar* is expressed differently in the two environments. The addition of shrimp in co-culture could introduce social stress to tilapia due to the increased stocking density, leading to competition among species (Milstein and Hernández, 2017). Although tilapia and shrimp occupy different niches, a certain degree of competition for food is always present in a co-culture system, even among species with different niches (Gonzales-

Corre, 1988). In aquaculture, the competition for resources could contribute to variability in body weight (Iung et al., 2020). Additionally, there is an assumption that in co-culture systems, when shrimp die or become moribund, tilapia may consume those shrimp (Juárez-Rosales et al., 2019). The social stress from increased density and the potential interactions with moribund shrimp in co-culture system could therefore lead to more variable growth of in Sukamandi tilapia compared to those in monoculture system.

Understanding the genetic correlation between growth and *LnVar* is essential for optimizing breeding programs to simultaneously improve both traits. The genetic correlations between *LnVar* and the growth traits W_4 and *DGC* were less than unity, 0.62 ± 0.12 and 0.40 ± 0.14, respectively. Genetic correlations between *LnVar* and growth trait W_4 and *DGC* from this study are in contrast with our estimate from GIFT tilapia grown in non-aerated ponds, where the genetic correlations between *LnVar* and growth trait W_5 and *DGC* were -0.52 ± 0.17 and -0.68 ± 0.12, respectively (Aththar et al., Chapter 2). *LnVar* and growth (W_4 and *DGC*) measured in Sukamandi tilapia are different traits compared to those measured in GIFT tilapia. *LnVar* and growth of Sukamandi tilapia were measured in brackish water ponds in the presence of shrimp whereas in GIFT tilapia, these traits were measured in freshwater ponds under monoculture conditions. These differences may explain the contrast in genetic correlations between *LnVar* and growth ($W_{4\text{-}5}$ and *DGC*). Additionally, the difference in the tilapia population and number of generations of selection is also a likely contributor to the difference in genetic correlation between *LnVar* and growth. In this study we used Sukamandi tilapia, which have been selected for growth in saline environments over five generations(Setyawan et al., 2022a), while the study by Aththar et al. (Chapter 2) used GIFT tilapia, which had undergone selection for growth over 17-18 generations (Mengistu et al., 2022).

The unfavorable genetic correlations between both *LnVar* and W_4 and *LnVar* and *DGC* were lower than unity. Therefore, growth and growth consistency, as measured by *LnVar*, could still be improved simultaneously by including *LnVar* and growth in the breeding goal with appropriate weights. We recommend that fish breeding programs collect repeated records on body weight and include both *LnVar* and growth in the breeding goal, assigning appropriate weights to each trait to enhance predictable fish growth in brackish water environments.

5.5 Conclusion

Sukamandi tilapia is able to thrive in co-culture with shrimps within brackish water environments with growth rates comparable to monoculture. We found heritable variation in *LnVar* for tilapia grown in the brackish water ponds. Surprisingly, *LnVar* was heritable in co-culture and not in monoculture, and we found moderate GxE between co-culture and monoculture. This suggests that genetic variation for growth consistency is expressed in the presence of shrimp. The magnitude of GxE for *LnVar* between co-culture and monoculture is higher than that for the growth parameters, suggesting that *LnVar* responds stronger to the environmental differences than growth. The genetic correlation between *LnVar* and growth of Sukamandi tilapia is less than unity, which supports the idea that *LnVar* and growth are different traits. To enhance predictable fish growth in the brackish water environment, we recommend that fish breeding programs collect repeated records on body weight and include both *LnVar* and growth in the breeding goal, assigning appropriate weights to each trait.

Acknowledgements

This study is part of the PhD project, funded by Koepon Foundation through grant number 4164016900 and Indonesia Endowment Fund for Education, Ministry of Finance Indonesia (LPDP - RI) grant number 20195221414387. We acknowledge the staff of Research Institute for Fish Breeding - Sukamandi and Technical Implementation Unit of Brackish water Culture - Karawang, Ministry of Marine Affairs and Fisheries Indonesia for providing the necessary help and facilities during the experiments.

Supplement 5.1 Cage distribution with co-culture and monoculture treatment in brackish water ponds for Sukamandi tilapia grow-out

We used two 25 x 50 m^2 brackish water ponds (Figure 5.3). In pond 1, we assigned 14 cages for co-culture treatment (1-14) and 13 cages for monoculture (28-40), while in pond 2, we assigned 13 cages for co-culture (15-27) and 14 cages for monoculture (41-54)

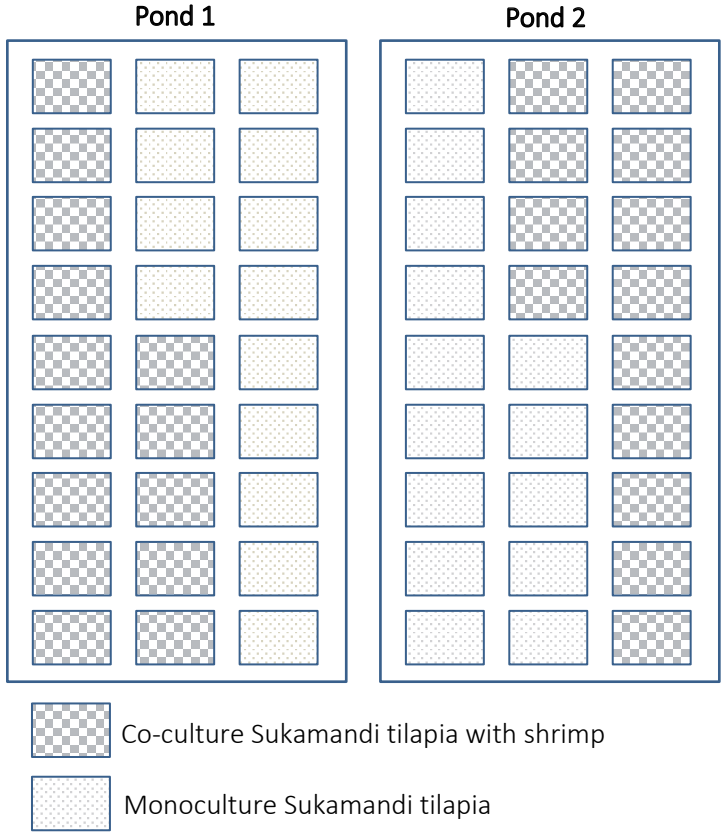

Figure 5.3 Cage distribution with co-culture and monoculture treatment in brackish water ponds for Sukamandi tilapia grow-out

Supplement 5.2 Growth trajectory of Sukamandi tilapia and GIFT tilapia

We used growth exponent and daily growth coefficient to plot growth trajectories of Sukamandi tilapia (f = 2.34 with DGC = 0.05 $g^{1/2.34}$/day, this chapter) and GIFT tilapia in non-aerated ponds (f = 1.77 with DGC 0.13 $g^{1/1.77}$/day, Chapter 2). We assumed the same initial weight of 25 grams. Daily weights were calculated as:

$$W_{it}(gr) = \left(W_1^{\frac{1}{fi}} + DGC_i * t\right)^{fi}$$

Where W_{it} is average weight for strain i at time point t, W_1 is the initial weight of 25 grams on day 0, f_i is the overall weight exponent of strain i, DGC_i is the average daily growth coefficient of strain i and t is the fish age. The GIFT tilapia grew at a faster rate than the Sukamandi strain. This is visually evident in the steeper slope of the growth curve for the GIFT tilapia compared to Sukamandi tilapia (Figure 5.4).

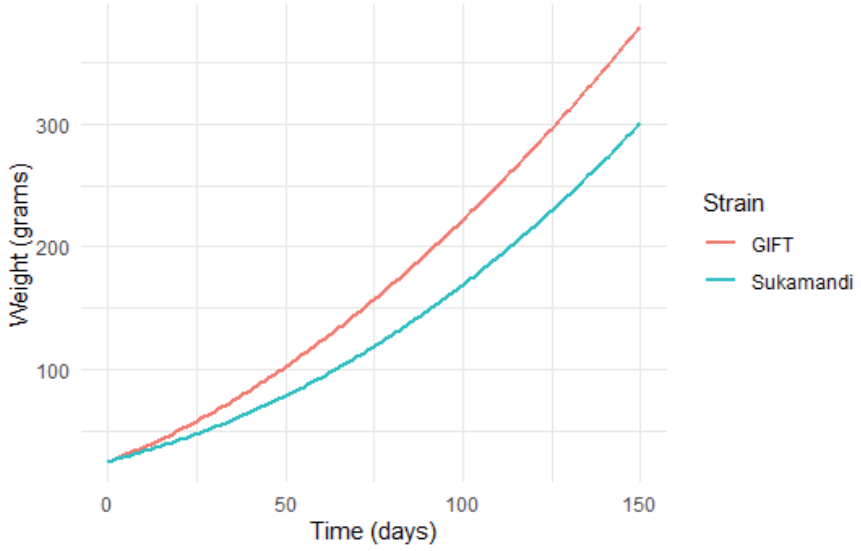

Figure 5.4 Average growth trajectories of Sukamandi tilapia in this chapter and GIFT tilapia in Chapter 2.

Chapter 6

General Discussion

In this chapter, I present a broader discussion on the concept of growth consistency as an indicator for resilience and its application in fish breeding programs. I start by describing the sources of stressors in aquaculture, followed by the discussion on growth consistency, resilience and environmental sensitivity. In the final section, I explore the application of *LnVar* in fish breeding programs.

6.1 Stressors in aquaculture

The dynamic environments and wide diversity of aquaculture production systems, along with the continuum from less intensive to more intensive production systems, can introduce various biotic and abiotic stressors. In aquaculture, stressors can be categorized into: 1) natural or environmental factors, including climate variability and water quality parameters (e.g., dissolved oxygen (DO), temperature, pH and salinity); 2) social factors, such as competition, density, aggressiveness; and 3) artificial (human-induced) factors such as cleaning, grading, handling, and transportation (Boyd, 2017; Tidwell, 2012; Sadoul and Vijayan, 2016; Wendelaar Bonga, 1997; McCormick et al., 1998; DiBattista et al., 2006). The effects of the stress response are generally detrimental because farmed fish cannot escape the continuous exposure to the stressors associated with aquaculture (Davis, 2006; Tort et al., 2011). Stressors can also be categorized as either chronic or acute. Chronic stressors induce a low intensity and slower-onset stress response but have a high energetic cost due to their duration, which can lead to distress and maladaptation, seriously compromising survival. In contrast, acute stressors are characterized by high severity, short duration, and abrupt onset of the stress response, based on the fight-or-flight reaction to ensure survival (Boyd, 2017; Schreck and Tort, 2016; Mateus et al., 2017).

Dissolved oxygen (DO) and salinity fluctuation are examples of the natural or environmental stressors. Recurrent hypoxia can affect fish productivity, including food intake, metabolic efficiency and growth (Brauner and Richards, 2020; Wang et al., 2023; Abdel-Tawwab et al., 2019;). DO is the environmental factor with the largest effect on growth rate in Nile tilapia (Mengistu et al. (2020b). However, many smallholder (Nile) tilapia farms do not use aerators, resulting in recurrent hypoxia in ponds. In an experiment by Mengistu et al. (2020a), GIFT tilapia were grown in non-aerated ponds with daily diurnal DO fluctuations (described in Chapter 2). DO levels fluctuated significantly throughout the day, ranging from above 3 mg/l during the daytime to below 1 mg/l at night, while in aerated ponds, DO levels remained consistently above 5 mg/l. Under hypoxia conditions (3 mg/l), Nile tilapia show significantly reduced growth compared to normoxia conditions (5 mg/l) (Mengistu et al., 2019). Harvest weight and daily growth coefficient (DGC) in non-aerated ponds were significantly lower than

in aerated ponds ($P < 0.01$) (Chapter 2). Non-aerated ponds also resulted in more variable growth pattern compared to aerated ponds. Furthermore, the feed conversion ratio (FCR) was higher in non-aerated ponds than in aerated ones (Mengistu et al., 2020a). The survival rate of fish was also lower in non-aerated ponds compared to aerated ponds.

Small-scale farmers in Indonesia are shifting from shrimp monoculture to shrimp culture in combination with other species, especially tilapia, using rotational cropping and co-culture of shrimp with tilapia. This shift follows severe disease outbreaks that have caused repeated failures in shrimp farming (Setyawan et al 2022; Modadugu and Acosta, 2004; Fitzsimmons and Shahkar, 2017; Wang and Lu, 2016; Wurmann et al., 2022; Martínez-Porchas et al., 2010). Shrimp farming is taking place in brackish water areas, such as the north coast of Java. To produce tilapia in this area, farmers need a fish that can be exposed to salinity fluctuations experienced in their ponds. Fluctuations in salinity cause osmotic stress and are a major limiting factor in aquaculture productivity in brackish water environments. In Chapter 4 and 5, Sukamandi tilapia were grown in non-aerated brackish water ponds in the coastal area of Indonesia, experiencing salinity fluctuations between 7 -25 ppt and daily DO fluctuations. We observed that Sukamandi tilapia growth was significantly better in brackish water ponds than in freshwater ponds (Table 4.2). The mass selection for growth in brackish water over five generations has already enhanced the salinity tolerance of Sukamandi tilapia (Setyawan et al., 2022a). Selection for growth in brackish water results in a more efficient osmoregulation with lower Na^+/K^+-ATPase concentrations, higher blood ion concentrations and higher *DGC* compared to selection for growth in freshwater (Setyawan et al., 2023).

The addition of shrimp in co-culture (Chapter 5), which led to the increased stocking density and interspecies competition, is categorized as a stressor from social factor. Although tilapia and shrimp occupy different ecological niches, a certain degree of competition for food is always present in co-culture system, even among species with distinct niches (Gonzales-Corre, 1988). In aquaculture, the competition for resources could contribute to variability in body weight (Iung et al., 2020). Additionally, it is assumed that in co-culture systems, when shrimp die or become moribund, tilapia may consume those shrimp (Juárez-Rosales et al., 2019). The difference in stressors magnitude between aerated and non-aerated ponds (Chapter 2) is expected to be greater than the difference between monoculture and co-culture treatments (Chapter 5). Therefore, the impact of environmental stressors is to be more noticeable, leading to differences in fish performance. Mean harvest weight (*HW*), survival and growth rate of GIFT tilapia were significantly lower in non-aerated ponds

compared to aeration (Mengistu et al., 2020a), while in Chapter 5, we found no significant differences in Sukamandi tilapia growth between co-culture and monoculture.

Routine procedures in aquaculture production such as handling and transportation are important elements in aquaculture. However, these activities are categorized as stressor from human-induced factor (Ashley, 2007). Handling stress may lead to physiological changes and impair growth in the aquaculture species. For instance, a study by Pickering et al. (1982) observed that a single handling stress in Brown trout resulted in several physiological changes lasting up to two weeks but did not affect growth rate. Furthermore, McCormick et al. (1998) showed that acute handling stressors decrease the growth of Atlantic salmon. To calculate *LnVar*, additional weight record between stocking and harvest are needed. We expect that stress from handling will increase *LnVar* and affect the comparison of *LnVar* between aerated and non-aerated as well as between co-culture and monoculture (Chapter 2 and 5, respectively). Automated phenotyping offers a non-invasive solution, making longitudinal measurements of individual fish more efficient and potentially more accurate by removing the stressors associated with handling (Li et al., 2020; Fu and Yuna, 2022), thus allowing for a more accurate measurement of *LnVar* in response to environmental stressors.

6.2 Growth consistency, resilience and environmental sensitivity

6.2.1 *LnVar* and resilience

Fish growth can indicate a fish's capacity to cope with environmental stressor. Growth results from a complex sequence of processes, starting with feed intake and proceeding through allocation of energy to muscle formation and size increase (Mommsen et al., 1999; Higgins and Thorpe, 1990). Stress can disrupt these processes, leading to reduced muscle growth (Barton, Bruce A., 2002; Guderley and Pörtner, 2010). In aquaculture, growth performance is typically assessed by recording body weight at stocking and harvest, which provides data on harvest weight and growth rates (Hopkins, 1992; De Graaf and Prein, 2005; Lugert et al., 2016). However, growth measured by harvest weight and growth rate alone may not fully capture the fish's capacity to respond to environmental stressors.

Exposure to repeated stressors in challenging aquaculture environments (i.e., daily diurnal DO fluctuations in Chapter 2 or salinity fluctuations in Chapter 4 and 5) along with the subsequent mechanisms or strategies to adapt, occurs continuously during the grow-out period. Fish may cope with stressors by reducing feed intake and will show compensatory

growth when favourable conditions are restored and food becomes available (Ali et al., 2003; Jobling, 2010)..

Resilience is defined as the ability of an animal to recover to its baseline state after stressors (Colditz and Hine, 2016). Friggens et al. (2022) emphasized that this capacity to bounce back from stressors typically occurs over a relatively short duration (green arrow, Figure 6.1). Since animals are repeatedly exposed to stressors, longitudinal data becomes essential for quantifying the ability of fish to consistently return to baseline levels after stressors (Friggens et al., 2022). In aquaculture production, longitudinal weight records can be used to measure fish ability to recover to baseline levels following stressors. In this thesis, we used natural logarithm of the variance of deviations from expected individual weights (*LnVar*), as a measure of growth consistency.

The different characteristics of traits affect the nature of deviations, as illustrated in Figure 6.1. For traits like growth in fish or pigs, the concept of an optimum trait applies. These traits are defined by a desired optimum value, where deviations can be both negative and positive (A). While trait like egg production in chickens, as represented in models (B), are considered maximum traits. This trait are characterized by a peak or maximum baseline, where the focus is on achieving the highest possible level. As a result, deviation from this peak can only be negative, indicating a drop from the maximum value (Berghof et al., 2024). In stressful environments, the nature of these deviations becomes particularly important. For optimum traits like fish growth, environmental stressors can cause negative deviations. Later, when conditions improve and food becomes available, fish may show compensatory growth, leading to a temporary acceleration in growth (Jobling, 2010) and resulting in positive deviations. On the other hand, for maximum traits, stressful environments are more likely to cause only negative deviations, as the stressors inhibit the organism's ability to reach or maintain peak performance levels, as shown by the reduction in egg production in commercial laying hens under heat stress (Mashaly et al., 2004).

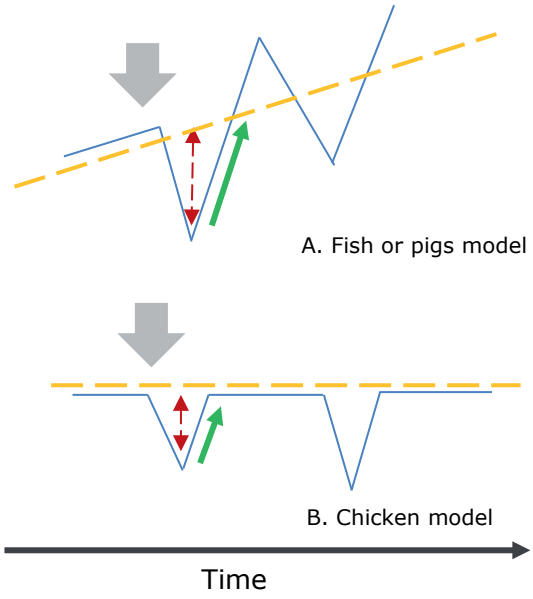

Figure 6.1 The performance of animals under stressors. Blue lines indicate animal performance, grey arrows indicate stressors, yellow dash lines indicate baseline performance, green arrows indicate the ability to recover to the baseline before stressor (resilience) with shorter arrows indicate more ability to recover, red lines indicate deviations between baseline state and post stressors level, black line indicates time.

In Figure 6.1 the stressors (grey arrow) causes a deviation (red arrow) from the expected performance (yellow line, Figure 6.1). This deviation from the expected performance at specific time point reflects the immediate impact of stressor on the animal and may indicate environmental sensitivity (Ros et al., 2004). $LnVar_{ind}$ could serve as an indicator of environmental sensitivity. It is essential to recognize that while *LnVar* for growth measures consistency in growth, it is not a direct measure of resilience.

6.2.2 *LnVar* based on individual and cohort approach

A baseline, the ideal performance trajectory in the absence of stressors, must be established to quantify deviations from the baseline and calculate *LnVar* (Scheffer et al., 2018). The baseline used to calculate these deviations should be less independent of environmental changes. In the previous study, Mengistu et al. (2022) used the deviation from the mean weight of the fish cohort to calculate *LnVar* ($LnVar_{coh}$). However, changes in the mean weight of the fish cohort depend on, besides growth of the fish, environmental conditions between time points that affect all fish in the cohort in the same manner. If there is a (negative) environmental effect, then all the fish are (negatively) affected by this environmental effect.

In addition, due to this (negative) environmental effect, there are also differences in the deviations from the baseline between individual fish. However, these individual variations are not visible if we use the cohort mean weight as the baseline. Instead of the cohort mean, we used non-linear regression to fit individual growth curves based on longitudinal weight measurements and used these growth curves as the baseline ($LnVar_{ind}$) (Chapter 2). The expected individual growth curve can show the individual variation of deviations due to the environmental effect. It can be predicted from the above that $LnVar_{ind}$ will show more variation than $LnVar_{coh}$. Indeed, $LnVar_{ind}$ showed higher phenotypic variance than $LnVar_{coh}$ in non-aerated ponds (1.563 and 0.988, respectively; Table 2.2).

The heritability estimate for $LnVar_{ind}$ in non-aerated ponds was 0.28. This was ~2 times higher than h^2 for $LnVar_{coh}$ in the same environment and ~4 times higher than $LnVar_{ind}$ in the other environment with aeration. For $LnVar_{coh}$ there was no difference in h^2 between non-aerated and aerated ponds (0.12 ± 0.05 and 0.10 ± 0.05, respectively). A higher heritability in non-aerated ponds would suggests that there is genetic variation for variability of growth measured with $LnVar_{ind}$ that comes to expression in the presence of environmental stressor from daily diurnal DO fluctuations in non-aerated ponds.

6.2.3 $LnVar_{ind}$ measure environmental sensitivity

$LnVar_{ind}$ measures the environmental sensitivity and therefore, $LnVar_{ind}$ could be an indicator for welfare. The sensitivity of animals to environmental stressors and their ability to adjust coping strategies in response to stressors are crucial aspects of welfare. Good animal welfare is characterized by a wide physiological and behavioural ability to anticipate and respond to environmental challenges (Korte et al., 2007). Fish with higher $LnVar_{ind}$ may indicate a greater ability to cope with stressors, with fluctuations in growth potentially reflecting an adaptation strategy to such stressors. For example, fish might reduce feeding activity as a coping mechanism during stress (Folkedal et al., 2012; Sadoul and Vijayan, 2016; Wendelaar Bonga, 1997). When conditions improve and food becomes available, fish may show compensatory growth by accelerating somatic growth temporarily (Ali et al., 2003; Jobling, 2010). On the other hand, a higher $LnVar_{ind}$ might also reflect reduced welfare if it indicates that an organism's coping mechanisms are failing to adapt to its environment, leading to suffering (Broom, 2008). Ensuring fish welfare in aquaculture is complex due to the numerous species-specific factors, including the welfare indicators, water quality parameters, environmental complexity and social behaviours of the animals (reviewed by Toni et al., 2019; Ashley, 2007). The relationship between $LnVar_{ind}$ and welfare is not yet fully understood. Further study is needed to investigate the correlation between $LnVar_{ind}$ and welfare-related traits, including

behavioural and physiological performance as well as health, which is a fundamental measure of welfare (Ashley, 2007). A study by Gorssen et al. (2024) found that in pigs, *LnVar* for growth was favourably correlated with reduced tail-biting, lameness and mortality, due to the increased uniformity within pen. Enhancing uniformity in fish may lead to more homogeneous size and decrease competition for food, resulting in a less feeding hierarchy and positively impacting animal welfare (Lines and Frost, 1999, Iung et al., 2020). Additionally, Berghof et al. (2019a) showed that *LnVar* for growth in chickens was predictive for lower lesion scores following avian pathogenic inoculation. Putz et al. (2019) also found favourable correlations between growth variation and mortality rates in pigs.

$LnVar_{ind}$ is useful for genotype by environment interaction (GxE) studies because $LnVar_{ind}$ measures environmental sensitivity. In Chapter 2, we observed a substantial GxE for $LnVar_{ind}$ between the aerated and non-aerated ponds (Table 6.1). The magnitude of GxE for $LnVar_{ind}$ between aerated and non-aerated ponds (r_g: 0.50) was higher than for $LnVar_{coh}$ (r_g: 0.80, Mengistu et al., 2022). In Chapter 5, we found moderate GxE for $LnVar_{ind}$ between co-culture and monoculture (r_g: 0.67) while GxE for growth and harvest weight was absent. Because $LnVar_{ind}$ measures environmental sensitivity it can help identify environments that are more demanding for fish. i.e., non-aerated ponds result in fish with higher $LnVar_{ind}$. Identifying these environments provides a basis for management interventions, such as aeration, which can help reduce $LnVar_{ind}$, leading to less feed waste and reduced environmental impact and potentially improving fish welfare. However, while improving aquaculture management may be necessary and even preferred for farmers, breeding for lower $LnVar_{ind}$ could offer cumulative and more sustainable benefits.

Table 6.1 Genetic correlations (r_g) of log transformed variance in ($LnVar_{ind}$), growth rate, and harvest weight and their standard errors (se) for tilapia between aerated and non-aerated and between monoculture tilapia and co-culture tilapia with shrimp

Traits	r_g aerated and non-aerated* (GIFT tilapia)	r_g monoculture tilapia and co-culture tilapia with shrimp** (Sukamandi tilapia)
$LnVar_{ind}$	0.50 (0.30)	0.67 (0.16)
Harvest weight	0.81 (0.30)[1]	0.96 (0.05)
Growth rate	0.78 (0.22)	0.99 (0.01)

[1]Mengistu et al., 2020a; * Chapter 2; ** Chapter 5

6.3 Application of *LnVar$_{ind}$* in fish breeding program

Determining the breeding goal is the first and important step for designing a breeding program. We calculated the economic value of *LnVar$_{ind}$* to determine its importance in breeding goals (Chapter 3). The economic values reflect the economic profit that can be obtained from genetic improvement of a trait within an aquaculture production system (Janssen et al., 2017a). The economic value of LnVar was calculated by considering the costs associated with reduced growth consistency. To investigate the economic response of including *LnVar$_{ind}$* in addition to *HW* in a breeding program to improve *HW* and *LnVar$_{ind}$* , we simulated two scenarios: one with selection index only on *HW* and another with index that included *HW* and *LnVar$_{ind}$*. Incorporating *LnVar$_{ind}$* into the index alongside *HW* in the breeding program to improve *HW* and *LnVar$_{ind}$* increased the response than the selection index only on *HW*, showing approximately 17% improvement in economic response.

An alternative approach to developing breeding objectives is the desired gains method (Nielsen et al., 2014), which considers the relative magnitudes of genetic gain desired in important traits (Sae-Lim et al., 2012). The goal of achieving desired gain is not necessarily to maximize profit from production but to produce a response that meets a specified objective.

A specific objective can be to align with the principles of sustainable production. Sustainable aquaculture based on three key aspects: economic, environmental, and social sustainability (Purvis et al., 2019; Garlock et al., 2024). For such an objective it is crucial to establish breeding goals that encompass environmental, and social sustainability in aquaculture, besides economic objectives (Janssen et al., 2017a; Besson et al., 2017; Olesen et al., 2003). *LnVar$_{ind}$* shows potential to contribute to economic, environmental, and social sustainability. Selecting fish with low *LnVar$_{ind}$* could improve economic results and contribute to economic sustainability (chapter 3). Incorporating *LnVar$_{ind}$* into the index alongside *HW* in the breeding program to improve *HW* and *LnVar$_{ind}$* increased the economic response than the selection index only on *HW* (Chapter 3).

Further, reducing *LnVar$_{ind}$* can benefit environmental impact and contribute to environmental sustainability. Fish with lower *LnVar$_{ind}$* show more consistent growth to those with higher *LnVar$_{ind}$* and therefore, reducing *LnVar$_{ind}$* leads to more predictable growth in fish. More predictable growth leads to more efficient feeding, reducing feed waste and gives benefits for environmental impact. Finally, *LnVar$_{ind}$* could be an indicator for welfare and therefore can contribute to social sustainability. However, the relationship between *LnVar$_{ind}$* and welfare is not yet fully understood (Section 6.2.3). Besides selection for *LnVar$_{ind}$*, the trait

can be used as a measure of environmental sensitivity in different production systems. $LnVar_{ind}$ can therefore be useful for assessing the improvements by aquaculture management.

I simulated a simplified Nile tilapia breeding program with the breeding goal of improving *HW* and $LnVar_{ind}$. The objective was to investigate the impact on selection response for production traits when focusing on environmental and welfare aspects. I used perspectives from economic, environmental, and social sustainability (welfare) to define the optimal desired gain for *HW* and $LnVar_{ind}$. In the economic scenario, the maximum response was assessed based on achieving the highest response for *HW* as the primary trait. In the environmental scenario, the focus was on maximizing the response for $LnVar_{ind}$, under the assumption that reducing $LnVar_{ind}$ would decrease feed waste and benefit environmental impact. Finally, in the social (welfare) scenario, given the uncertain relationship between $LnVar_{ind}$ and welfare, $LnVar_{ind}$ was kept constant while evaluating the response in *HW*.

The responses to selection were calculated in SelAction v2.1 (Rutten et al., 2002). The breeding goal (*H*) was defined as:

$$H = W_{HW} * A_{HW} + W_{LnVar} * A_{LnVar}$$

where, *W* is the breeding goal weight for *HW* or $LnVar_{ind}$. A is the additive genetic value for *HW* or $LnVar_{ind}$. I used genetic parameters of GIFT tilapia grown in non-aerated ponds with daily diurnal DO fluctuations (Table 2.2 and 2.4; Chapter 2). The weights W_{HW} and W_{LnVar} were varied between +1 to -1 to generate an ellipse, representing selection responses. In the simulation, 120 females were mated with 40 males to produce 120 full-sib families. From each family, 40 fish (20 females and 20 males), totalling 4,800 fish, were considered as selection candidates. From these candidates, 40 males and 120 females were selected as parents for the next generation. The proportion of selected male and female parents are 0.0167 and 0.05, respectively. The selection process used all available information, including individual performance as well as data from full and half-sibs.

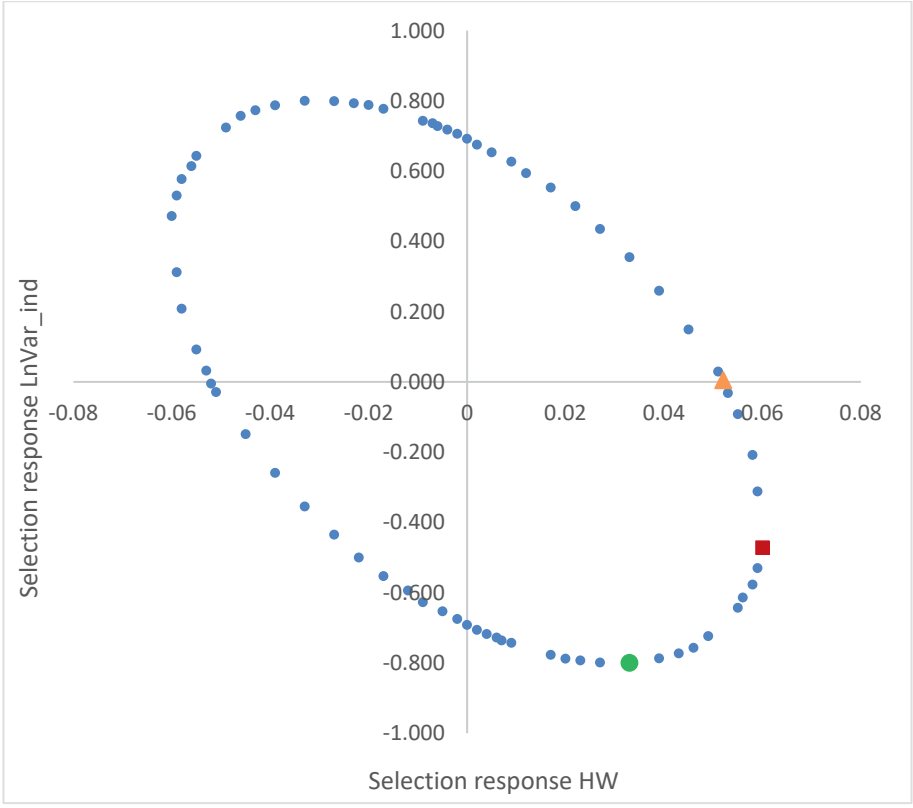

Figure 6.2 Selection responses for harvest weight and $LnVar_{ind}$ with the weighing for of harvest weight set to +1 and −1, and for $LnVar_{ind}$ varying between −1 and +1 in a tilapia breeding program. The figure shows the response ellipse of $LnVar_{ind}$ and *HW* with a genetic correlation of −0.45. Red box indicates the economic scenario, Green circle indicates the environmental scenario and orange triangle indicates the welfare scenario.

Figure 6.2 showed the response of $LnVar_{ind}$ and *HW* and indicates the responses for the economic, environmental and welfare scenarios. For the economic scenario, the maximum response for *HW* was found at weighing value of +1 for *HW* and -0.01 for $LnVar_{ind}$ (red box in Figure 6.2; Table 6.2). The trait response for harvest weight and $LnVar_{ind}$ were 0.060 kg and -0.47 unit $LnVar_{ind}$, respectively. The maximum response for $LnVar_{ind}$, for the environmental scenario was found at weighing value of +1 for *HW* and -1 for $LnVar_{ind}$ (indicated by the green circle in Figure 6.2). The trait response for *HW* and $LnVar_{ind}$ were 0.033 kg and -0.80 unit $LnVar_{ind}$, respectively. If we want to maximize reduction in $LnVar_{ind}$ to give maximum benefit for environmental impact, there is a reduction of 0.027 kg (45%) in *HW* compared to production scenario. For the welfare scenario, if we keep the $LnVar_{ind}$

constant then trait response for *HW* is 0.052 kg which is a reduction of 0.008 kg (13 %) in *HW* compared to the production scenario. (indicated by orange triangle in Figure 6.2).

Table 6.2 The response of *HW* and $LnVar_{ind}$ in economic, environmental and welfare scenario

Scenario	Response	
	HW (kg)	$LnVar_{ind}$ (unit $LnVar_{ind}$)
Economic	0.060	-0.47
Environmental	0.033	-0.80
Welfare	0.052	0.00

The paradigm of breeding goals in aquaculture have shifted toward balanced breeding goals that aim for the simultaneous improvement of production, efficiency, and functional traits, with growth still remaining a central objective (Næve et al., 2022; Houston et al., 2022; Janssen et al., 2017b; Olesen et al., 2003). A balanced breeding goal can be defined by assigning weights to each trait based on derived economic values (EV) and nonmarket value (NV) (Olesen et al., 2000; Olesen et al., 2003). NV reflects the extent to which farmers or breeders are willing to lose selection response in production traits to improve or maintain functional traits, thereby directly integrating aspects such as environmental impact and welfare into selective breeding (Nielsen et al., 2005; Nielsen et al., 2006). This simulation showed that using a desired gain approach, breeders can include $LnVar_{ind}$ in breeding goals that could lead to simultaneous improvement of both production and environmental impact or welfare in aquaculture.

6.4 Concluding

The dynamic environments and wide diversity of aquaculture production systems, along with the continuum from less intensive to more intensive production systems, can introduce various environmental stressors. With repeated exposure to stressors and the physiological adaptation in fish, therefore, longitudinal records of growth are necessary to capture the fish's capacity to respond to stressors. In this thesis, we used natural logarithm of the variance of deviations from expected individual weights ($LnVar_{ind}$), as a measure of growth consistency, to capture the fish's ability to handle stressors.

$LnVar_{ind}$ is important be included in fish breeding programs because improving $LnVar_{ind}$ leads to more predictable growth in fish. More predictable growth results in more efficient feeding practices, further reducing feed waste that gives benefit for environmental impact. In addition, including $LnVar_{ind}$ in breeding goals, alongside growth as the main trait, results in positive economic response.

LnVar$_{ind}$ is a measure of environmental sensitivity, which could make *LnVar*$_{ind}$ an indicator for welfare. The sensitivity of animals to environmental stressors and their ability to adjust coping strategies in response to stressors are crucial aspects of welfare. However, the relationship between *LnVar*$_{ind}$ and welfare is not yet understood, and this should be investigated. *LnVar*$_{ind}$ is also useful for GxE studies because *LnVar*$_{ind}$ measures environmental sensitivity and can help identify environments that are more demanding for fish. i.e., non-aerated ponds result in fish with higher *LnVar*$_{ind}$. Identifying these environments provides a basis for management interventions, such as aeration, which can help reduce *LnVar*$_{ind}$, leading to less feed waste and reduced environmental impact. However, while improving aquaculture management may be more preferable for farmers and more cost-effective than breeding, breeding for lower *LnVar*$_{ind}$ could offer cumulative and more sustainable benefits.

Calculating *LnVar*$_{ind}$ requires longitudinal records of individual fish weight. Automated phenotyping can make longitudinal measurements of individual fish more efficient and potentially more accurate. This technology has been developed and applied across various aquaculture species, including salmon, catfish, tilapia, and seabream. The advancement of automated phenotyping technology will significantly facilitate the application of *LnVar*$_{ind}$ in fish breeding programs.

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Summary

The dynamic environments and wide diversity of aquaculture production systems, along with the shift from less intensive to more intensive practices, can introduce environmental stressors. With repeated exposure to stressors, longitudinal data becomes essential for quantifying the ability of fish to consistently return to baseline levels. In aquaculture production, longitudinal weight records can be used to measure fish ability to recover to baseline levels following stressors. In this thesis, I used natural logarithm of the variance of deviations from expected weights (*LnVar*), as a measure of growth consistency. The aim of this thesis is to improve methods for measuring resilient growth and to provide knowledge for improving the resilient growth in tilapia through breeding program. The specific objectives of this thesis are: 1) To develop a novel measure of *LnVar* using individual growth curves as expected performance, 2) To estimate the genetic correlation between *LnVar* and growth in freshwater ponds with or without daily diurnal dissolved oxygen fluctuations, 3) To investigate the economic value of *LnVar* and the potential of economic gain from *LnVar* using selective breeding, 4) To estimate the genotype-by-environment interaction for growth between freshwater ponds and brackish water ponds and the impact of the presence of shrimp on these genetic parameters and 5) To estimate the genetic parameters for *LnVar* and the correlation between *LnVar* and growth in brackish water ponds with strong salinity and temperature fluctuations.

In **Chapter 2**, *LnVar* was calculated by fitting the expected individual growth curve based on longitudinal observed weight ($LnVar_{ind}$). This growth curve represents the ideal growth trajectory for fish in the absence of stressors. We used a dataset of GIFT tilapia that were grown in either an aerated or non-aerated freshwater pond and in which weight was measured at five time points during the grow-out period. The results showed that $LnVar_{ind}$ was found to be highly heritable in the more challenging environment and this can be exploited by selective breeding. The negative correlation between $LnVar_{ind}$ and growth implies that selection for growth may also improve $LnVar_{ind}$. Genetic correlation of $LnVar_{ind}$ between aerated and non-aerated ponds was 0.50, suggesting that genetic improvement for growth in the aerated environment will not automatically lead to improved $LnVar_{ind}$ in the non-aerated environment. Therefore, it is beneficial to incorporate information from records of relatives in the non-aerated ponds if the breeding program is intended for this environment. We recommend measuring $LnVar_{ind}$ through repeated weight records and based on the individual expected growth trajectories in fish breeding programs to simultaneously improve growth and resilient growth. Further, in this summary, I used the term *LnVar* to refer to $LnVar_{ind}$.

Determining the breeding goal is the first and important step for designing a breeding program. We calculated the economic value of *LnVar* to determine its importance in breeding goals. *LnVar* was calculated by fitting the expected individual growth curve based on longitudinal observed weight. In aquaculture practices, feed requirements are predicted based on expected fish weight estimated from periodic sampling of groups of fish. It can be hypothesized that deviations of actual weight from expected weight will lead to economic losses. In **Chapter 3,** we derive the economic value of *LnVar* and explore the potential of economic gain from reducing *LnVar* using selective breeding. To calculate the economic value of *LnVar,* we define the effect of fluctuations in fish growth as the economic loss resulting from feed waste, growth deficiency and feed saving. The resulting economic value (EV) for *LnVar* is 0.043 US$/unit LnVar/kg. production. The breeding program to improve *HW* and *LnVar* with the selection index only on *HW* showed a total economic response of 0.110 US$/kg per generation, whereas incorporating *LnVar* into the index alongside *HW* increased the response to 0.122 US$/kg, showing approximately 11% improvement in economic response. Further, to investigate the economic response of including *LnVar* alongside *HW* in a breeding program to improve both traits, we simulated two scenarios: one with selection index including only *HW,* and another with index that included *HW* and *LnVar*. Incorporating *LnVar* into the index alongside *HW* in the breeding program to improve *HW* and *LnVar* increased the economic response by more than 11% compared to the selection index including only *HW*. Therefore, we recommend that fish breeding programs collect repeated records of body weight and include *LnVar* in the breeding goal.

In Indonesia, recurrent farming failures due to disease outbreaks have driven shrimp farmers to develop co-culture between shrimp and tilapia. Shrimp farming takes place largely in brackish water areas, such as the north coast of Java. To produce tilapia in this area, farmers need a fish that can be exposed to salinity fluctuations experienced in their ponds. The Research Institute for Fish Breeding (RIFB) Indonesia has been conducting a small-scale breeding program for salinity tolerance using the Sukamandi tilapia to develop a fast-growing tilapia with good growth over a range of fluctuating salinities in brackish water ponds. A freshwater nucleus and evaluation breeding program is the simplest strategy to implement but requires knowledge of the extent of the genotype-by-environment (GxE) interaction between fresh and brackish water environments. In **Chapter 4**, we investigate the impact of salinity on genetic parameters and the presence of GxE between brackish water and freshwater ponds in Sukamandi tilapia. The results showed that brackish water ponds provide better support for fish growth, resulting in higher growth performance. We conclude

that there is substantial GxE interaction for growth between brackish water and freshwater. We recommend that a breeding program for salinity-tolerant tilapia with a safe, stable, low-risk, and bio-secure freshwater nucleus should incorporate sib information on growth performance in brackish water.

Furthermore, to increase production in brackish water environments, fish farmers need resilient tilapia capable of consistent and predictable growth performance. In **Chapter 5**, we estimated genetic parameters for growth and for *LnVar* in the Sukamandi tilapia in brackish water. We produced 102 tilapia families and randomly assigned fingerlings to grow-out in co-culture with shrimps or to grow-out in monoculture. Results showed that Sukamandi tilapia is able to thrive in co-culture with shrimps within brackish water environments, achieving growth rates comparable to monoculture. We found heritable variation in *LnVar* for tilapia grown in the brackish water ponds. We found moderate GxE between co-culture and monoculture. This suggests that genetic variation for growth consistency is expressed in the presence of shrimp. The magnitude of GxE for *LnVar* between co-culture and monoculture is higher than that for the growth parameters, suggesting that *LnVar* is more responsive to the environmental differences than growth. The genetic correlation between *LnVar* and the growth of Sukamandi tilapia is less than unity, which supports the idea that *LnVar* and growth are different traits. To enhance predictable fish growth in the brackish water environment, we recommend that fish breeding programs collect repeated records on body weight and include both *LnVar* and growth in the breeding goal, assigning appropriate weights to each trait.

In Chapter 6, I present a broader discussion on the concept of growth consistency as an indicator for resilience and its application in fish breeding programs. I start by describing the sources of stressors in aquaculture, followed by a discussion on growth consistency, resilience and environmental sensitivity.

Appendices

About the Author
List of Publications
Training and Supervision Plan
Acknowledgements

About the Author

Muhammad Hunaina Fariduddin Aththar (**Farid**) was born on 6 April 1984 in Madiun, Indonesia. he pursued a bachelor's degree in Aquaculture at IPB University in Bogor from 2002 - 2007, with a thesis titled *'Effectiveness of the B-actin promoter of medaka (Oryzias latipes) using the hrGFP (humanized Renilla reniformis green fluorescent protein) gene marker in walking catfish (Clarias gariepinus).* After graduation, Farid joined the Fish Genetics and Breeding Laboratory in the Department of Aquaculture at IPB University as a junior researcher. In 2008, he began working as a researcher at the Research Institute for Freshwater Aquaculture (RIFA) under the Ministry of Marine Affairs and Fisheries (MMAF) of Indonesia. Farid was involved in several breeding programs for carp, Nile tilapia, and gourami in Indonesia. Notably, the Rajadanu carp and BEST (Bogor Enhanced Strain Tilapia) tilapia, developed through these breeding programs, were officially released by the Minister of Marine Affairs and Fisheries in 2009 and 2015, respectively. From 2010 -2012, he also served as Head of the Research Station for Freshwater Fish Germplasm and Genetics at RIFA.

In 2012, Farid began his MSc studies in Aquaculture at IPB University and graduated with Cum Laude honors in 2014. This program was funded by a Master's Scholarship from the Center of Education – MMAF. His MSc thesis was titled *'Phenotypic and Early Development Performance Analysis of Sumatra, Java, and Kalimantan Snakeskin Gourami (Trichopodus pectoralis).'* After completing his MSc, he resumed his role as a researcher at MMAF. From 2014 - 2019, he worked on research and domestication efforts for several fish species, including the Asian red-tail catfish (*Hemibagrus nemurus*), barred loach (*Nemacheillus fasciatus*), snakehead fish (*Channa micropeltes*), and *Ompok hypopthalmus*. In 2014, he assisted the assessment of fish breeding programs in Indonesia as part of the Fisheries and Aquaculture Food Security in Indonesia (FAFI) project, a collaboration between Wageningen University and Research (WUR) and MMAF. From 2015 to 2019, he also served as a member of the Technical Team for Feed Biosafety Assessment at the Indonesia National Biosafety Commission under the Ministry of Agriculture. In 2018, he received the Honorary Medal "Satya Lencana Karya Satya X" from the President of Indonesia for his 10 years of service at MMAF.

In 2019, Farid began his PhD project at the Animal Breeding and Genomics - WUR. His PhD was funded by the Doctoral Fund Scholarship from the Indonesia Endowment Fund for Education (LPDP) under the Ministry of Finance, and the Koepon Foundation. His PhD research results are presented in this thesis, titled *'Breeding for Resilient Growth in Tilapia,'* supervised by Hans Komen and John Bastiaansen. Following a reorganization in the Indonesian government, Farid joined the National Research and Innovation Agency (BRIN) as a researcher in 2022. Farid can be reached via email: **mh.fariduddin.aththar@brin.go.id or farid.aththar@gmail.com**

List of Publications

Publications

Setyawan, P.*, **Aththar, M. H. F**.*, Imron, I., Gunadi, B., Haryadi, J., Bastiaansen, J. W. M., Camara, M. D., & Komen, H. 2022. Genetic parameters and genotype by environment interaction in a unique Indonesian hybrid tilapia strain selected for production in brackish water pond culture. Aquaculture, 561, 738626.
*equally contributed

Gustiano, R., Arifin, O.Z., Subagja, J. Kurniawan, K., Prihadi, T.H., Saputra, A., **Aththar, M. H. F**., Cahyanti, W., Prakoso, V.A., Radona, D., Kusmini, I.I., Kristanto, A.H. 2023. The Success of Freshwater Aquaculture Program: Nile Tilapia or "Nila" Culture In Indonesia. Zuriat 34 (2), 56-68.

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Contributions to conferences

Aththar, M. H. F., Bastiaansen, J. W. M., Komen, H. 2024. Improving growth rate estimation of Nile tilapia (*Oreochromis niloticus*) by non-linear regression. WIAS Science Day 2024. *Oral presentation*

Aththar, M. H. F., Setyawan, P., Bastiaansen, J. W. M., Komen, H. 2023. Resiliensi Ikan untuk akuakultur berkelanjutan di Indonesia [Fish resilience for sustainable aquaculture in Indonesia]. Kongres Pelajar Indonesia di Belanda. Den Haag, the Netherlands. *Oral presentation*

Aththar, M. H. F, Komen, H., Bastiaansen, J. W. M. LnVar from individual growth as potential indicator of resilience in Nile tilapia (Oreochromis niloticus). WIAS Annual Conference 2023: Surpassing Boundaries-Going one step ahead, 17-17. *Oral presentation*

Aththar, M. H. F., Setyawan, P., Komen, H. Bastiaansen, J. W. M. 2022. Heritable indicators for resilience of tilapia grown in brackish water ponds; The International Symposium on Genetics in Aquaculture; Puerto Varas - Chile ISGA XIV 2023. *Oral presentation*

Bastiaansen, J. W. M., **Aththar, M. H. F**., Setyawan, P., Komen, H. 2023. Heritabilities and correlations of linear, exponential, and asympotic measures of fish growth. ; The International Symposium on Genetics in Aquaculture; Puerto Varas - Chile

Aththar, M. H. F., Bastiaansen, J. W. M., Komen, H. 2023. Breeding program for resilient tilapia (Oreochromis niloticus) in Indonesia; Wageningen Indonesia Scientific Exposure (WISE); Wageningen - The Netherlands. *Oral presentation*

Aththar, M. H. F, Setyawan, P., Imron, I., Gunadi,B., Camara, M., Komen, H., Bastiaansen, J. W. M. 2022. The potential indicator for resilience of Sukamandi strain tilapia grown in brackish water ponds. 12th World Congress of Genetics Applied for Livestocks Production, Rotterdam. *Oral pitch presentation*

Setyawan P., **Aththar, M. H. F.**, Imron I., Gunadi B., Haryadi J., Bastiaansen, J. W. M., Camara M., Komen H. 2022. Genotype by environment interaction for productivity traits of a hybrid tilapia in fresh and brackish water ponds. Aquaculture Europe 2022. Rimini, Italy.

Aththar, M. H. F., Setyawan, P., Bastiaansen, J. W. M., Camara, M., Komen, H. 2021. Genetic Parameters of Reproductive Traits for Tilapia Oreochromis Niloticus in Brackish Water and Freshwater. Aquaculture Europe 2021, Madeira Portugal. *Oral presentation*

Aththar, M. H. F., Setyawan, P., Bastiaansen, J. W. M., Komen, H. 2021. Genetic parameters for growth and genotype by environment interaction of Nile tilapia in freshwater and brackish water. WIAS Annual Conference 2021, the Netherlands. *Oral presentation*

Setyawan, P., **Aththar, M. H. F**., Imron I., Gunadi B., Haryadi J., Yu X., van den Burg S. W. K., Bastiaansen J., Camara M., Komen H. 2021. Sustainable breeding program of tilapia for small-scale brackish water farmers. Week of Indonesia-Netherlands Education and Research (WINNER). The Netherlands.

Aththar, M. H. F., Bastiaansen, J. W. M., Komen, H. Improving growth and survival of tilapia in brackish water. WIAS Annual Conference 2020: Frontiers in Animal Sciences, the Netherlands. *Poster presentation*

Training and Supervision Plan

Education and Training	Year	ECTs
A. Basic Packages		**2.0**
WIAS Introduction Day	2019	0.3
Scientific Integrity and Ethics in Animal Sciences	2020	1.5
B. Disciplinary Competences		**19.0**
WIAS PhD Proposal	2020	6.0
Genetic Improvement of Livestock	2020	6.0
Estimation of Genetic Parameters and Breeding Value	2019	1.2
Genotype by Environment Interaction, Uniformity and Resilience	2020	1.5
Mixed Linear Model	2021	0.6
Advanced Statistics course Design of Experiments	2022	0.8
Genomic prediction considering admixed populations and GxE	2022	0.9
Simulation of breeding programs with Modular Breeding Program Simulator (MoBPS)	2023	0.6
AquaIMPACT training course on Genomic Innovations for Aquaculture Breeding	2023	1.0
C. Professional Competences		**7.0**
Project and Time Management	2020	1.5
The Essentials of Scientific Writing and Presenting	2020	1.2
Scientific Writing	2020	2.0
Efficient Writing Strategies	2021	1.3
The Final Touch	2023	0.6
D. Societal Relevance		**1.0**
Communication with the Media and the General Public	2022	1.0
E. Presentation Skills		**4.0**
Genetic parameters of reproductive traits for tilapia (Oreochromis niloticus) in brackish water and freshwater; Aquaculture Europe; Madeira - Portugal	2021	1.0
The potential indicator for resilience of Sukamandi strain tilapia grown in brackish water ponds; The 12th World Congress on Genetics Applied to Livestock Production (WCGALP); Rotterdam - The Netherlands	2022	1.0

Heritable indicators for resilience of tilapia grown in brackish water ponds; The International Symposium on Genetics in Aquaculture; Puerto Varas - Chile	2022	1.0
Breeding program for resilient tilapia (Oreochromis niloticus) in Indonesia; Wageningen Indonesia Scientific Exposure (WISE); Wageningen - The Netherlands	2023	1.0
Improving growth rate estimation of Nile tilapia (Oreochromis niloticus) by non-linear regression; WIAS Science Day; Reehorst Ede - The Netherlands	2024	1.0
F. Teaching Competences		**1.0**
Teaching assistant in course (ABG-31306 Genetic Improvement of Livestock)	2021	1.0
Total		**33**

Acknowledgements

Finally, my five years of hard work have paid off. I am incredibly happy to have completed my PhD at Wageningen University & Research. Throughout this journey, I have learned so much, not only within the academic world but also beyond it. I realize now that a PhD is not just an academic and scientific journey but also a path of self-discovery. Without the enormous support of my supervisory team, family, friends, and colleagues, I would not have been able to complete this journey. I want to express my gratitude to everyone who played a role in my PhD journey, though I find this part tricky to put into words.

First, I would like to extend my sincere thanks to the Indonesia Endowment Fund for Education, Ministry of Finance (**LPDP - Lembaga Pengelola Dana Pendidikan**, Kementerian Keuangan Indonesia) for awarding me the Doctoral Fund Scholarship. This scholarship paved the way for my PhD journey. Beyond its objective of improving human resources by sending many awardees to world-class universities, this scholarship also reflects Indonesia's commitment to contributing to global scientific development. I would also like to thank **Wageningen University & Research** for allowing me to pursue my PhD degree, and to the **Koepon Foundation** for funding the experiments in Indonesia. Furthermore, I am grateful to the **Ministry of Marine Affairs and Fisheries (MMAF), Indonesia**, and the **National Research and Innovation Agency (BRIN)** for permitting me to carry out my PhD project.

A special thank you goes to the most important person in my PhD journey: my promotor, **Hans Komen**, and my daily supervisor, **John Bastiaansen**, who have been immensely supportive, friendly, and always available to provide assistance. **Hans**, thank you for giving me the opportunity to start my PhD at ABG – WUR. Your recommendation helped me a lot to secure the PhD funding from LPDP. Your guidance and tireless support throughout my PhD have been invaluable. Your long experience and expertise in aquaculture and breeding programs ensured the quality of my work. I particularly appreciated your critical and detailed points of view. I thoroughly enjoyed our discussions, especially during the monthly meetings and in the final stages of my PhD. You taught me not just about science but also about life, recommending great books and offering travel tips. Our last trip to Chile for ISGA was unforgettable, especially with your meticulous planning. Lastly, as you always said, "There's always a good reason for a drink." I will miss our drinks and gatherings at your warm-hearted home. We both completed our tasks this year—enjoy your retirement, Hans!

John, thank you for being my daily supervisor over these past five years. Your prompt and valuable feedback throughout my PhD journey, along with your support during the application for LPDP funding and throughout the COVID-19 pandemic, when we were running two major experiments in Indonesia, was indispensable. I greatly appreciate your patience in explaining complex topics and your thorough attention to detail, which helped me immensely during this PhD. During our meetings, we didn't only talk about science and my research, but also about family and life matters. Those conversations were invaluable in maintaining a healthy work-life balance. I will miss our weekly Tuesday meetings. We've

shared several memorable trips, from MedAid data collection in El-Campello, Spain, to the EAS in Madeira, ISGA in Chile, and data collection at Kingfish Zeeland. I look forward to future collaborations with you, and I hope we can enjoy Sate together in Indonesia, just as we did when we first met during your lecture in Sukamandi, Indonesia, in 2018

Robbert Blonk and **Edward Schraam**, thank you for connecting me to ABG - WUR through the Fisheries and Aquaculture Food Security in Indonesia (FAFI) project, a collaboration between Wageningen University & Research and MMAF. The continuation of this collaboration motivated and partly funded my PhD journey. I fondly remember our "Pecel Lele" street food dinner in Bogor on the first day of the FAFI workshop and the subsequent training and travel within the project. **Kasper Janssen**, thank you for our conversation with John during the last FAFI training, which encouraged me to pursue a PhD at ABG – WUR. I would also like to thank to **Amien** and **Febri** from the FAFI project.

Priadi, your dedication to the two major field experiments in Indonesia is truly remarkable—thank you! These experiments formed two key chapters of my thesis. We managed the first data collection during the COVID-19 pandemic, and although I couldn't join the second experiment, your efforts ensured its success. I look forward to many future collaborations, now that we are working together at BRIN. I would also like to thank the BRPI Sukamandi staff (**Lamanto, Bisri, Ifan, Imron, and Bambang**) and the BLUPB Karawang staff, especially **Deni Sugianto**, for their support during the experiments in Indonesia. **Mark Camara**, you joined ABG during the COVID-19 pandemic. Thank you for your help in my early steps learning ASReml and for organizing tilapia student meetings to plan and execute the field experiments in Indonesia. Your contribution as a co-author in Chapter 2 of this thesis is greatly appreciated. Samuel, thank you for guiding me through the dataset from your experiment at WorldFish, which I used to write another chapter of this thesis. Congratulations on your new role at WorldFish! I also extend my thanks to **John Benzie, Hugues de Verdal,** and **Charles Rodde** for giving me the opportunity to work with the WorldFish dataset from Malaysia.

Thank you to the ABG secretaries, **Lisette** and **Fadma**, who helped me immensely during my PhD, as well as **Nicole, Marielle** and **Danielle** from ABG secretary and **Paddy** from WIAS. **Rosilde**, thank you for ensuring all the financial matters related to the project ran smoothly. I would also like to thank **Martien Groenen**, the former chair of ABG, and **Mario Calus**, the current chair, for their support throughout my PhD.

A big thank you to my paranymphs, **Renzo** and **Diyon**. **Renzo**, my PhD buddy, night shift companion (until 2022), and meme buddy. Our corridor chats always made me feel welcome at ABG – WUR. So I start my PhD with you as PhD buddy —thank you for making the early part of my PhD go smoothly and you are now becoming my paranymph during my defense as my completion of PhD trajectory. And I won't forget, Forza Inter Milan! **Diyon**, thank you for your immense help during my defense preparation, and for all the enjoyable moments we shared, whether traveling, exploring culinary delights, cycling, watching movies and football match, playing FIFA and even a small catch-up for coffee in city center. I will also

never forget the delicious home-cooked meals you and your wife, Deila, prepared. Thank you for everything. All the best for you and **Deila**!

I would like also to thank to all Aquabreeding members: To my Koko, **Xiaofei**—we worked side by side to make the second experiment in Indonesia a reality, and I am grateful for your help with the sample shipments. I hope we can continue to stay in touch via Skype or WeChat to update our life and future collaboration. **Benan**, we shared the same promoter and daily supervisor. Thank you for all the chats and discussion we had, the nice trip for data collection within your project in El - Campello, Spain and trusting me as your paranymph during your PhD defense. I hope we can enjoy Turkish food together again as we did during ISGA in Rotterdam. All the best for you, Seyda and Dunya. **Pauline**, we shared the same corridor and PhD experiences during my early days. I'm always impressed by the beautiful eel pictures in your presentations. All the best to you, Roy, and Pepijn. **Enyam**, thank you for all the discussion related to the fish physiology, and our late-evening corridor chats that helped relieve my stress during the final stretch of my PhD. Good luck with the completion of your thesis. You can do it, bro! **Fasil**, good luck with your new postdoc position at Roslin, and thank you for trusting me as the paranymph during your defence. **Meilisa**, I enjoyed our discussions on the economic aspects of breeding and our collaboration on future project ideas. **Yuuko**, thank you for being great co-paranymph for Benan and also our trip to Chile during ISGA was amazing! **Wout**, thank you for the discussions and chats in Radix and also during the travel for the data collection to Kingfish. Great to have you as our "city guide" in Middelburg and your nice recommendation to visit Teyler Museum in Haarlem. I would also like to extend my thanks to **Arjan**, **Hendrik-Jan, Bram, Manu, Tiago** for all the discussions during focusgroup meeting or in Radix and also to **Chantal** for organizing group "wandelling" enjoying the Levadas in Madeira with Xiaofei and the new experience of treating horse during the visit to your horse stable with Enyam and Fasil.

Thank you also to my former and recent corridor mates: **Haniel Cedraz** for the laughs and chats during your stay in ABG, and keeping up with our life updates through Instagram; **Lim-lee**, for our daily encouraging chats; **Dries**, I hope we cross paths again as you will start to work on shrimp disease in Indonesia; **Helene**, for all the chats we had, for your support, and the invitations for group dinners. Good luck with your postdoc in the US. **Eugene**, for the corridor chats, your delicious homemade bread and cookies, and the reminders about group lunch; **Wen-Ye**, good luck with planting trees online and on your cupboard, and remember, you have a talent for bike trading! to **Gibbs, Aixin, Zhuoshi, Tzhayri, Pedro, Tobias, Junxin, Olivia, Anne** (please let me know when you have a plan to revisit Sukabumi, I will show you the best tempe culinary), **Huifang** and **Wenqi**, all the best for the rest of your PhD journey.

I would also like to thank the GIL teaching team of 2022; **Henk Bovenhuis, Han Mulder, Harmen,** and **Anouk** for giving me the support with the new experience for me on teaching. **Harmen**, don't mix up the scarf and wind jacket since we have the same ones :). Anyway, thank you for all the chats that we had in Radix during my PhD. **Anouk**, you recently finished your PhD with cum laude—how impressive! I would like also to thank to **Bart Ducro, Piter Bijma, Richard Croijmans, Ibrahim, Carolina, Istvan, Rayner, Gerbrich, Jani, Biaty, Matias,**

Pascal, Jan-Erik, Alfonso and **Jan van der Lee**. You are all made ABG feel like home for me. Also to **Bert** and **Kimberley**, I will always cherish our trips for data collection to El-Campello and Zeeland, respectively.

I would like to thank all my supervisors from my bachelor's and master's studies, as well as my research at MMAF, for their guidance and motivation, which have helped me reach this level. Pak **Rudhy Gustiano**, your guidance and support as my mentor from the beginning of my career at MMAF have been invaluable. Your meticulous planning and the high standards you set have greatly helped me as I continued in academia. More than that, you've continuously motivated me to pursue my PhD. Pak **Komar Sumantadinata** and Pak **Alimuddin**, thank you for introducing me to aquaculture and fish genetics during my bachelor's studies and for laying the knowledge foundation in fish breeding and reproduction. Pak **Zairin Junior**, your motivation to never give up in the pursuit of scientific passion, as well as your support during my LPDP grant application, meant a great deal to me. I would also like to extend my thanks to Pak **Anang Hari Kristanto,** Pak **Triheru Prihadi,** Pak **Brata Pantjara,** Pak **Adang Saputra,** Pak **Toni Ruhimat,** Bu **Norma** for their support during my time in RIFA – MMAF and pre-departure preparation to start my PhD in WUR.

Now, I will switch to Bahasa Indonesia so I can convey the essence and meaning more precisely.

Terima kasih untuk keluarga besar Indonesia saya di Wageningen. Perjalanan PhD ini terasa lebih bermakna dan berwarna berkat adanya teman-teman yang akhirnya menjadi saudara di Wageningen. Terima kasih atas kumpul-kumpulnya, makan-makan, jalan-jalan, sepedaan, nonton film bareng, nonton dan bola, badminton serta semua aktivitas-aktivitas lain.

Ucapan terima kasih untuk tetangga terdekat, Herenstraat 21 family, **Alim, Ayu, Abi, Alesha**. Untuk teman-teman seperjuangan dan keluarga yang memulai PhD di WUR 2019: **Steven**, terima kasih buat rutinitas makan siang bareng, update progress sekolah, undangan makan malam dan kiriman coklat (all the best buat Steven, **Litha dan Gavriel**); **Mas Ludi,** buat ngobrol, diskusi dan sharing di akhir-akhir penyelesaian thesis (all the best buat Mas Ludi – Mba Lia serta keluarga). Untuk Herenstraat family: **Mas Firin – Ceu Windi, Bang Emil – Mba Lina, Kang Edwin – Teh Sandra, Mba Mayang (**dan **Jimly)**. Untuk keluarga Herenstraat 21-2: **Mas Ahmad, Abah Mugni, Wahdan, Mba Ayu, Yayang, Arief, Shesar dan keluarga**, terima kasih atas momen-momen yang mengesankan, berbagi cerita, makan bareng, nonton bareng. Tali silaturrahmi ini telah menjadi tali persaudaraan. Untuk tetangga beda gedung, **Mas Obi** dan **Mba Zidnie** (terima kasih untuk undangan makan-makan dan sarana luapan jokes bapak-bapak. Sukses selalu untuk Obi dan Zidnie!).

Terima kasih untuk teman-teman dan keluarga yang sudah kembali ke Indonesia: **Pak Eko, Mba Andra, Ali** (trip ke Luxembourg sangat berkesan dan matur nuwun Pak Eko sudah mempercayakan saya menjadi paranymph saat defence), **Pak Dikky** (yang masih rajin jenguk ke Wageningen, untuk obrolan dan diskusi serta oleh-olehnya), **Mas Fahriz dan Mba Fifi**, **Mas Sahri - Mba Ami** (terima kasih Mas Sahri untuk atas corridor chatnya di Radix), **Mas Nove -**

Mba Lia (masakan kambing khas van Uvenweg dan rekomendasi perburuan sepeda tidak pernah gagal)**, Kukuh** dan **Bib Yaqin** (momen ketika kita makan Kibbeling dan kentang dekat Ivan sangat berkesan dan tidak akan pernah terlupakan)**, Mas Darmanto – Mba Nadya** (matur nuwun atas undangan makan masakan Padang dan BBQ-nya), **Mas Hardi dan Mba Eva** (terima kasih atas racikan kopi barista pak haji andalan Wageningen).

Untuk grup jalan-jalan, kuliner dan nonton bareng (**Diyon - Deila, Imam – Sita - Henzie, Mas Danny** - **Mba Rika – Emily** (Matur nuwun Mas Danny sudah dipercaya menjadi paranymph pas defense-mu)**, Mba Fiametta - Mas Fahmi** (Forza Milan!) **– Atika**, **Mas Anto** - **Mba Nila**), terima kasih semuanya atas momen-momen Bahagia jalan-jalan ke Keukenhof, Lisse, nonton film di Utrecht dan Amsterdam, kuliner Korea di Rotterdam dan makan di Lapek Jo yang hampir nyasar ke pempek Elysha.

Terima kasih banyak **Arul,** sobat curhat 24/7 jam yang sewaktu-waktu muncul di Herenstraat 21-2 untuk sekedar ngobrol santai atau bikin makanan, selalu tulus dan ga pernah ada pamrih. Terima kasih untuk semua momen jalan-jalan bareng. Terima kasih juga sudah dipercaya menjadi saksi ikatan cintamu di Gementee Wageningen (All the best for you, **Betul and Zakir**)**, Shiddiq - Ika**, terimakasih untuk momen makan-makan di Plantsoen dan road trip ke South Tirol dan Dolomites Winter 2022 yang amazing. Untuk **Koh Sam**, terima kasih buat sayur lodeh dan martabak manis yang tidak pernah gagal. Good luck buat tenure tracknya Koh! **Mas Dana - Mba Diana**, terima kasih banyak sudah rutin menyuplai makanan selama periode akhir menyelesaikan thesis; Untuk KK Sulsel Wageningen (**Tante Titi, Om Gerard, Ulil, Andra – Istri - Faqih, Vivi, Nunik, Anwar, Daus**), terima kasih atas kebersamaan dan persaudaraannya. Lebaran seperti serasa di Indonesia. Untuk **Wagenesia Fietsers** (**Mas Danny, Koh Sam, Diyon, Fahmi, Nove, Shiddiq, Mas Firin, Mas Anto, Kukuh, Mas Radar**), terima kasih sudah berbagi *cycling trip* dari mulai short trip bridge to bridge Wageningen, coffee trip Kasteel Dorwerth, lunch trip Arnhem, sampai long trip ke Kleve, Kinderdijk, Delft, Den Haag (Lapek Jo trip), Drielanden punten, Aachen dan Veluwe National Park. Terima kasih buat **Awang** dan **Robbith** atas obrolan-obrolan santai kita di luar nalar. Untuk teman-teman **grup FIFA – WUR** (**Edwin, Sunu, Ngakan, Diyon, Ahmad, Erbi, Reyza, Mas Ikhsan, Maslim, Aby, Ibnu, Desta, Rizal Makarim)**, terima kasih sudah membuat malam-malam yang sepi menjadi meriah dan bergairah.

Terima kasih juga kepada **Pak Agus**, Atdikbud KBRI Den Haag dan **Bang Ronald**, Atase Imigrasi KBRI Den Haag yang sudah sering menjenguk kami di Wageningen. Terima kasih juga buat **Haniswita** (bumbu gulai padangny ajaib! Sukses dan lancar selalu ya bro utk PhD-nya), **Mas Sunu – Mba Wida**, **Rizal - Isna, Iqbal – Putri, Margi – Nindya, Mba Puspi – Mas Faiz, Koh William, Firdaus – Nadiya, Mas Andi – Mba Ruth, Pak Gede – Mba Nima** atas kebersamaannya. Ucapan terima kasih juga saya sampaikan kepada teman-teman BRIN - WUR (**Mas Ludi, Mas Priadi, Ceu Miwa Miranti, Teh Intan, Mas Ikhsan, Mas Agusman, Mba Susi**), sampai bertemu kembali dan berkolaborasi di Indonesia.

Selanjutnya, ucapan terima kasih saya sampaikan kepada teman-teman eks Kelti Genetika dan Reproduksi BRPBATPP Sempur yang telah membersamai saya melakukan kegiatan riset

sebelum berangkat sekolah, **Pak Jojo, pak Otong, Bu Irin, Vitas, Mas Deni, Mba Lia dan Mba Sri**. Ucapan terima kasih juga saya sampaikan ke **Pak Iman Hidayat, Bu Evi Arida, Bu Delicia** dan **Mas Rivo** serta teman-teman BOSDM - BRIN, yang telah banyak membantu proses awal-awal integrasi KKP ke BRIN serta memberikan dukungan untuk kelancaran proses tugas belajar saya di WUR.

Terima kasih buat kedua orang tuaku tercinta, **Bapak** dan **Ibu** (**Pak Tamrin** dan **Bu Hana**) yang selalu mendukung tanpa pamrih dan tidak pernah mengekang keinginanku dalam mencari ilmu meskipun akhirnya sejak lulus SD sudah jauh dari rumah bahkan akhirnya sekarang tinggal jauh dari bapak ibu. Tidak pernah lekang dari ingatan ketika bapak ibu pertama kali mengantarkan sekolah ke Jombang. Matur nuwun juga sampun jenguk ke Belanda pas Winter 2022. Melepas rindu setelah beberapa tahun tidak bertemu sambil liburan Europe trip seperti oase di tengah padang pasir, memberikan tambahan energi dan semangat dalam perjalanan sekolah ini. Pak, Bu… Alhamdulillah, akhirnya hari ini putramu bisa mencapai gelar akademik tertinggi. Semuanya tidak lepas dari jerih payah, usaha, doa dan dukungan Bapak Ibu sampai hari ini. Semoga lulusnya kuliahku di Wageningen ini bisa menghadirkan kebahagiaan buat Bapak dan Ibu…

Terima kasih untuk **Papa dan Mama** di Selayar (**Pak Syaid** dan **Bu Hami**) atas semua doa, dukungan dan perhatian selama perjalanan sekolah di Belanda ini. Meskipun tidak banyak kesempatan untuk bersama dan bertemu, tetapi pada beberapa momen ketika bisa ngobrol dengan Papa dan Mama sampai pagi di Bogor, juga ketika menjenguk saya dan Vina ke Jakarta sewaktu pulang ke Indonesia dan Road trip ke Serang sangat berkesan.

Terima kasih buat adikku satu-satunya, **Tifa**. Selalu sabar dan semangat terus menemani bertumbuhnya Xander dan Akyfa. Jangan pernah lelah untuk belajar. Untuk **Riky**, terima kasih banyak sudah selalu ada untuk Tifa, Akyfa, Xander serta Bapak dan Ibu di Semarang. Untuk adik-adikku, **Aa Baktiardo – Fafa dan Dede Yoga - Novi**, terima kasih selalu atas doa dan dukungannya. Tidak lupa juga buat semua keponakanku, **Xander, Akyfa, Uel, Aqa, Fathan dan Naya**. Semoga gelar doktor ini bisa menjadi pemacu semangat untuk tidak pernah lelah belajar dan mengejar cita-cita.

Terakhir, buat yang tercinta **Apriana Vinasyiam**, Vina. Tidak ada kata-kata yang bisa mengungkapkan seberapa besar rasa sayang dan rasa terima kasihku ke kamu. Perjalanan sekolah ini tidak mungkin bisa dimulai kalau tidak ada dorongan untuk juga bisa menemanimu sekolah di Belanda. Meskipun pada perjalanannya tidak semudah yang kita bayangkan tapi Alhamdulillah kita berdua bisa melewati dan menyelesaikannya. Sekolah bareng di Belanda juga memberikanku banyak pembelajaran dan menjadi proses pendewasaan buat aku serta akan menjadi bekal sampai akhirnya nanti kita bisa menua bersama. Terima kasih terus selalu menemaniku dengan sabar. Terima kasih untuk bisa membagi waktu dengan baik antara sekolah dan rumah, hal yang sampai saat ini aku masih takjub. Sebagaimanapun padatnya jadwal untuk menyelesaikan sekolah masih bisa masak uenak, mengerjakan beberapa pekerjaan rumah. Terima kasih juga untuk bisa memberikanku kesempatan sepedaan, nonton bola dan pergi ke museum untuk melepas penat. Selama

hampir 8 bulan terakhir ini kita terpisah jarak dan waktu, kamu tidak pernah putus memberikanku semangat, dorongan dan dukungan untuk bisa menyelesaikan thesis. Sekali lagi, terima kasih… Yang, Alhamdulillah Desember ini aku selesai sekolah dan bisa menemanimu lagi di Bogor untuk petualangan-petualangan kehidupan selanjutnya. Semoga Allah selalu membersamai kita dengan ridlo dan petunjuk-Nya.

Finally, I may have forgotten to thank someone and realize that others haven't been specifically mentioned in this acknowledgment. I apologize for this oversight, and believe me, my appreciation for you is as great as it is for those mentioned above.

Wageningen, Autumn 2024
Farid

Colophon

The research described in this thesis was financially supported by the Indonesia Endowment Fund for Education, Ministry of Finance, Indonesia (LPDP - Lembaga Pengelola Dana Pendidikan, Kementerian Keuangan Indonesia) and the Koepon Foundation. The program was additionally funded by grant KB-35-001-001 under the Wageningen University and Research "Food Security and Valuing Water"program, Ministry of Agriculture, Nature and Food Quality and Covid-19 Compensation Fund for PhD candidate – *National Programma Onderwijs*, the Netherlands.

Cover design by Fajri Maulana and Farid Aththar (Gencraft)

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