Selection for growth of Nile tilapia *(Oreochromis niloticus* L.) in low-input environments

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Harrison Charo Karisa

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"Ninayaweza mambo yote katika Yesu anitiaye nguvu…" Philippians 4:13

Contents

Chapter 1

General Introduction

1. Aquaculture and capture fisheries

While the global human population continues to increase rapidly (UN, 2005), the world's fishing areas have reached their maximal potential for capture fisheries production (FAO, 2004). To meet the increased demand for food fish, aquaculture production should increase by 50 million Metric tons by 2050 (Tacon and Forster, 2001). Aquaculture is currently the fastest growing segment of food production in the world and by 2002 was contributing approximately 30% by weight of fish and shellfish consumed worldwide (FAO, 2004). However, present aquaculture technology requires large amounts of fish meal and oil from wild caught fish which leads to further depletion of natural stocks (Marra, 2005; Tacon, 2004). In Africa and the rest of the developing world, fish catches hardly meet the demand as a resource for human food, leaving no room for aquaculture's requirements. It is clear that the expansion of aquaculture under the current production conditions is not sustainable.

Alternative protein sources for fish feed, and the production of herbivorous and omnivorous fishes, such as carp and tilapia, which feed at lower trophic levels have been suggested as solutions to aquaculture's reliance on fish meal (El-Sayed, 1999; Kautsky et al., 1997; Williams et al., 2000; Azim et al., 2004). However, the overall efficiency of fish reared in fertilized ponds also needs to be improved. Studies have shown that whereas about 15-30% of the nutrient input in pellet-fed pond systems is converted into harvestable products (Acosta-Nassar et al., 1994; Gross et al., 2000), only 5-15% of the nutrient input in fertilizer-only pond systems is converted to harvestable products (Schroeder et al., 1990; Edwards, 1993). To improve the overall nutrient use efficiency of fish in fertilized ponds and reduce cost of production, efficient breeding programs are crucial. The genetic improvement of livestock and crops has for a long time led to dramatic increase in yields. Selective breeding in aquaculture is still at infancy compared to livestock farming. However, because higher selection responses to growth have already been reported for several fish species (Olesen et al., 2003), there are high prospects for successful selective breeding in aquaculture.

2. Nile tilapia in aquaculture

Tilapias are among the most important warm water fishes used for aquaculture production and originate from Africa and the Middle East (Fryer and Iles, 1972). Among tilapiines, the Nile tilapia, *Oreochromis niloticus* L. and its hybrids, is the most important cultured fish

species. It is becoming an increasingly important food fish in many parts of the world (Pullin, 1997). Although, principally herbivorous in nature (Moriarty, 1973; Moriarty and Moriarty, 1973), *O. niloticus* can feed on a wide variety of natural food organisms found in organically fertilized ponds (Yashouv and Chervinski, 1961; Bowen, 1982) as well as on artificial feeds. Majority of the culturing of *O. niloticus* is carried out in the tropics in semi-intensive small-holder farms. Due to high cost of feeds (Liti et al., 2005) and limited protein sources, most farmers cannot afford supplementary feeds. Therefore, whereas commercial tilapia farms report yields of 10 000- 15 000 kg/ha/yr, small-scale tilapia fertilized earthen pond farms report yields of approximately 500kg/ha/yr (Machena and Moehl, 2001).

2.1 Selective breeding of Nile tilapia

A number of selection experiments and breeding programs aiming at increased growth rates in ponds have been conducted for *O. niloticus* (Hulata et al., 1986; Brzeski and Doyle, 1995; Eknath et al., 1993; Bentsen et al., 1998; Bolivar and Newkirk, 2002). These selective breeding programmes have typically been done in favourable environments where growth is high. In these environments, fish receive high protein supplementary feeds formulated commercially from fish meal and oil. However, in Asia and Africa, Nile tilapia is farmed under variable small-scale production systems with a wide array of resources as pond inputs: grasses, weeds, chicken droppings, cow dung, rice bran, and leftover food, which add to the diversity of tilapia farming systems. In the presence of genotype by environment interaction (GXE), a strain that has been genetically improved through selection in a favourable environment may not be necessarily good for less favourable conditions. Large GXE necessitates the development of strains highly adapted to specific environments, and a small interaction permits the development of strains that will perform well in a broad spectrum of environments. Little is known about the degree of GXE in tilapia.

3. Selection for growth in diverse environments

To produce strains that can perform satisfactorily over diverse environments, it is important to know the conditions in which selection should be carried out. Jinks and Connolly (1973) and Jinks and Pooni (1988) showed that sensitivity (i.e. the difference between measurements of a genotype in two environments) is reduced by selection upwards in a bad environment and by selection downward in a good environment, both of which are cases of antagonistic selection

i.e. the selection changes the character in the opposite direction. Based on the Jinks-Connolly rule, and a review of a number of selection experiments, Falconer (1990) hypothesized that to increase the mean performance of a trait over a wide range of environments, selection should be done in a sub-optimal environment since this is likely to reduce the environmental sensitivity of the genotypes. However, to select effectively in sub-optimal environments, several issues need to be addressed. Traditionally, most selective breeding takes place in high- rather than low-yielding environments. This has been based on the assumption of higher heritabilities for traits in high-yielding than in low-yielding conditions, because of more efficient control of the environment and better expression of genetic differences in high-yielding environments (van Oosterom et al., 1993). Other issues revolve around the correlated responses to selection for growth in low-input environments and genotype by environment interactions.

Most sub-tropical regions and highland areas in the tropics frequently go through low temperatures which hamper tilapia growth. The study presented in this thesis was carried out at Abbassa, east of the Nile Delta in Egypt, which experiences cold spells during winter. The optimal temperature for growth of most tilapiine species is between 25-28 °C and feeding stops below 20 °C (Wohlfarth and Hulata, 1983). Nile tilapia cannot survive temperatures less than 10-12 °C for more than a few days (Chervinski, 1982). Tolerance to low temperatures is dependent upon environmental and genetic effects, history of the fish, as well as fish health and nutrition status (Cnaani et al., 2000). The genetic basis of cold tolerance is poorly understood and little is known about the differences in cold tolerance within and between tilapiine species. Wohlfarth et al. (1983) and Cnaani et al. (2000) studying some tilapia species and their hybrids found that a large component of the trait's variance was a result of dominance effects. However, in Nile tilapia, Tave et al. (1989, 1990) and Behrends et al. (1990) suggested that cold tolerance shows mainly additive genetic variance. Knowledge of the genetic nature of cold tolerance could aid in improving the tolerance of Nile tilapia to low temperature and extend its grow-out period.

4. Interdisciplinary collaborative research

This thesis is a part of Wageningen University's Interdisciplinary Research and Education Fund project entitled "Optimization of nutrient dynamics and fish for integrated agriculture-

aquaculture systems" (INREF-POND). The main objective of INREF-POND is to quantify and optimize changes in nutrient dynamics in integrated agriculture-aquaculture (IAA) farming systems after the introduction of selected fish breeds. The IAA systems are characterized by low level of inputs, and traditional, indigenous breeds of fish, cattle, pigs, goats, sheep, and poultry. Due to the exchange of resources between subsystems at the farm, it is necessary to optimize the efficiency of exchange of resources and nutrients between different components of the IAA. To achieve this, the INREF-POND project is organized following a multi-level approach: farm, pond, and fish levels (Figure 1). The farming system research (encompassing the combined terrestrial-aquatic system) aims at fine-tuning the fish, livestock, and crop compartments of the integrated farming system. This focuses on the role of IAA systems in livelihood improvement strategies as well as on the environmental issues related to the farming system. At the pond system level, the research aimed at optimizing the role of the pond in nutrient trapping and fish production, and minimizing the environmental impacts of the pond. This research involved the characterizaton of high and low input fish production systems.

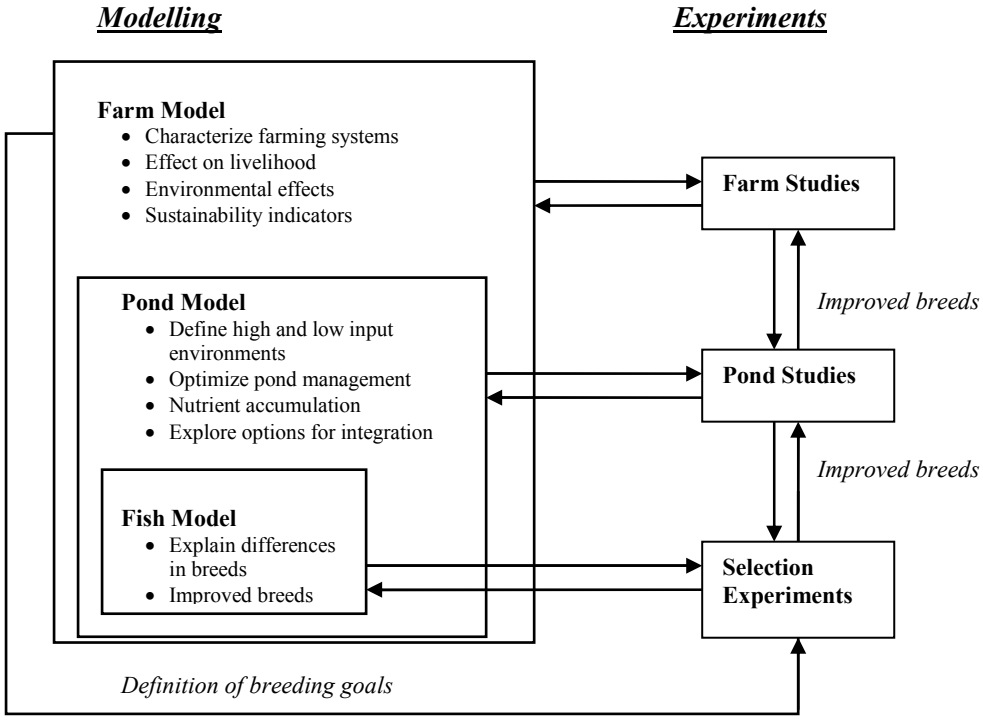

Figure 1. Summary of research in the INREF-POND project (Adapted from INREF Pond Progress report 2003)

The present study, which is at the fish level, aims at improving the fish for optimal performance in their environments. It involved selective breeding for improved growth of Nile tilapia in low- and high-input pond systems. The performance of the two resultant strains over a range of environments was evaluated. This project was implemented collaboratively between Wageningen University (WU), The WorldFish Center (WFC), and Can Tho University (CTU), Vietnam. The research presented in this thesis was carried out at WFC's Regional Centre for Africa and West Asia, Abbassa, Egypt.

5. Aims and outline of the thesis

This thesis aims at studying the feasibility of selective breeding for the improvement of growth of Nile tilapia in low-input extensive farming conditions, and at determining the effects of selection on other performance traits. It further intends to support the production of improved broodstock and fish seed for the resource poor fish farmers and thereby contribute to reduced malnutrition and poverty alleviation.

Chapter II presents results of an experiment that was carried out to determine the optimal conditions for early rearing of tilapia fry in hapa-in-pond systems. **Chapters III** and **IV** deal with cold tolerance of Nile tilapia juveniles. The heritability of cold tolerance and the effect of size on cold tolerance of juveniles are presented in **Chapter III**, and the effects of environmental factors and diet on cold tolerance are presented in **Chapter IV**. Results of the main selection experiment are presented in **Chapters V** and **VI. Chapter V** gives the estimates of the heritability and response to selection for body weight and the heritability for survival till harvest in low-input fertilized ponds. It shows the feasibility for carrying out selection in fertilized ponds without supplementary feeds. **Chapter VI** presents an analysis of the heritability of body measurements, gut length, and correlated responses to selection for growth. In **Chapter VII**, the selection lines are tested across a range of potential farm environments to investigate the presence of genotype by environment interactions. Lastly, **Chapter VIII** discusses the feasibility for selection in low-input conditions, and the practical lessons learnt from this research in view to carrying out selection for low-input and heterogeneous pond environments.

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Chapter 2

Genetic and environmental factors affecting growth of Nile tilapia (*Oreochromis niloticus*) juveniles: modelling spatial correlations between hapas

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Abstract

The aim of this study was to quantify the environmental and genetic effects on early growth of Nile tilapia, *Oreochromis niloticus*, in hapa-in-earthen pond systems. In a pilot study, we grew swim-up fry with or without supplementary feed in hapas suspended in fertilised ponds at 5, 10, 15, and 20 fry/m^2 densities. In the main experiment, we reared swim-up fry from 25 full-sib families separately for 42 days at 15 fry/m^2 density in hapas suspended in two earthen ponds. Hapas were arranged in two column arrays along the sides of the ponds. Ponds were fertilized daily with chicken manure. In addition, fry in one column in each pond were fed twice daily on 40% protein pelleted feed. Results from the pilot study indicated significant effects of stocking density and treatment. In the main experiment, the dietary treatment effect was not significant but there were large differences in growth between ponds. Mean body weight at 42 days was 1.7 g in pond A, and 0.4 g in pond B. Heritability (h^2) of 42-day fry body weight estimated from the whole data set, using a univariate model, was 0.01±0.06. The bivariate heritability estimates were 0.59±0.19 in pond A and 0.05±0.11 in pond B. The common environmental / hapa (c^2) effects were 0.14±0.06 and 0.29±0.07 in respective ponds. We found significant positive spatial autocorrelation (P=0.02) indicating resemblance in growth of fry in neighboring hapas. Analysis of environmental variables showed that the two ponds differed significantly in dissolved oxygen. The low genetic correlation (r_g = -0.27±0.69) between body weights of fry in both ponds might therefore suggest genotype by environment interactions for tolerance to low dissolved oxygen in Nile tilapia juveniles.

Keywords: Nile tilapia; *Oreochromis niloticus*; hapa-in-pond; heritability; spatial autocorrelation

1. Introduction

Apart from mechanisms that contribute directly to maintenance of genetic variance, the conditions under which variance components are estimated may influence the assessment of heritability (Simons and Roff, 1994). Response to selection can be increased by reducing environmental variation between families through techniques of rearing and management (Falconer and Mackay, 1996). However, effective management of the rearing environment may not be possible without understanding the underlying environmental factors influencing the expression of traits. This is because fish performance may vary even in apparently similar

environments. For example, while rearing tilapia fry in seawater pools fertilised with chicken manure, Ernst et al. (1989) found considerable variability in fish performance among pools despite identical management methods.

In fish breeding programs, members of a full-sib family usually share a common tank or hapa prior to tagging and communal testing. In Nile tilapia, *Oreochromis niloticus*, hapas suspended in fertilized ponds are increasingly used for rearing of fry until tagging size or grow-out stage (Little et al., 2003a). Apart from feeding on a variety of supplementary feeds, tilapias feed on fertilizer-induced phytoplankton (Moriarty and Moriarty, 1973; Bowen, 1981). As a result, fry in hapas-in-pond systems may be reared with a range of feeds and pond inputs varying in nutrient content. When reared in different environments, genotypes may exhibit differences in growth performance. However, studies on performance of fry in hapa-in-ponds have so far only considered the effect of density and nursing duration (Sanches and Hayashi, 1990; Little et al., 2003b).

For convenience of monitoring and identification of families, hapas are often arranged in rows over the pond. The micro-environment created within hapas may increase resemblance of individuals reared in the same hapa. When ponds are heterogeneous, for example with respect to nutrient availability, the spatial arrangement of hapas may also create an environmental correlation among neighbouring units. At present, it is not clear whether or how the spatial arrangement of hapas in the pond and the environment within hapas influence fry performance.

The aim of this study was therefore to quantify the genetic and common environmental effects on early rearing stages of *O. niloticus* in hapa-in-pond nursing conditions. We reared tilapia fry from full-sib families in two different dietary treatments and ponds to determine (i) the effects of rearing environments on heritability estimates of early growth, and (ii) whether and how spatial arrangement of hapas in the ponds affects early growth of *O. niloticus*.

2. Materials and Methods

2.1 Pilot experiment: Effect of fish density and dietary treatment on fry growth

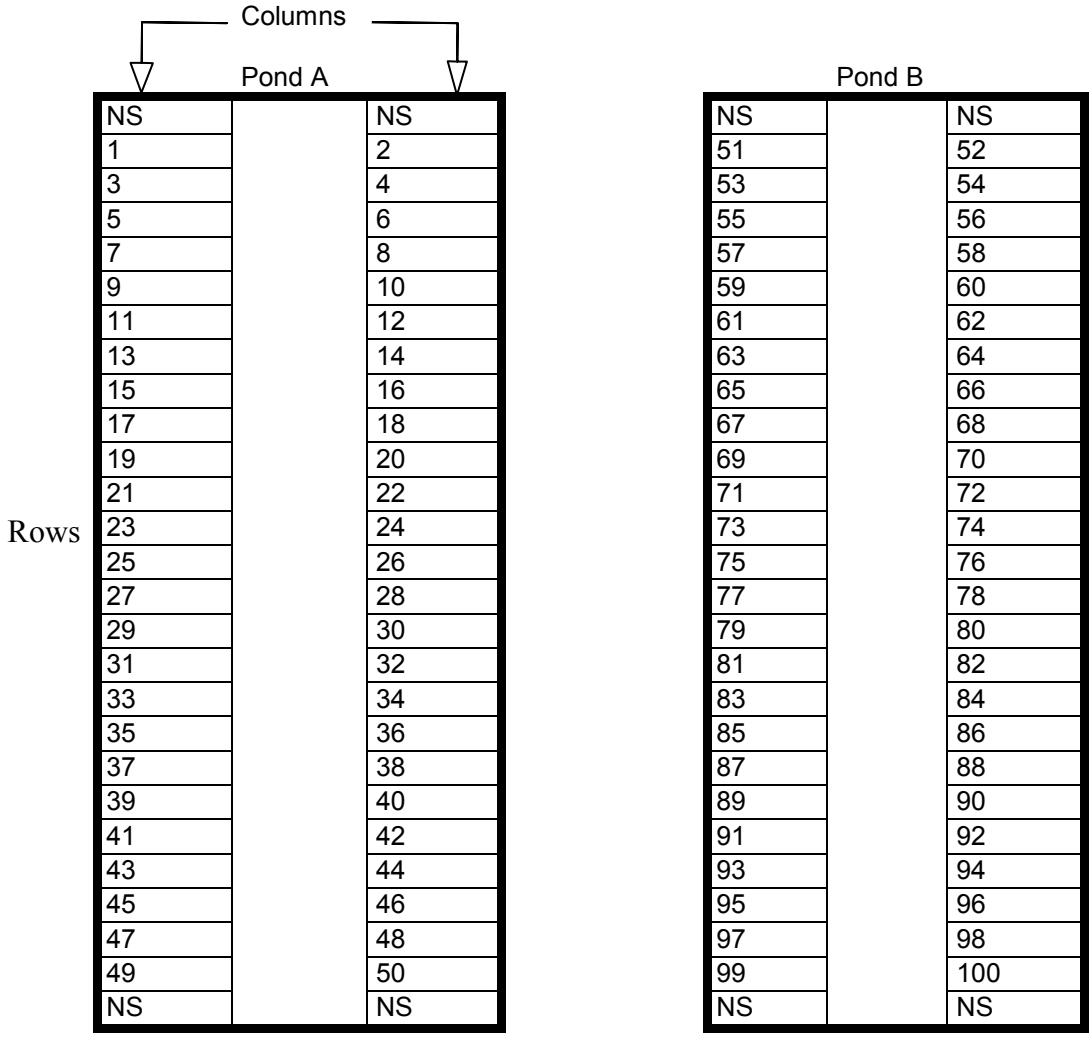
First we carried out a pilot experiment to compare growth of fry at different densities in fertilized ponds with or without supplementary feed. The specific objective of this study was to determine densities at which swim-up fry would grow at comparable rates in natural food and supplementary-fed hapa conditions. Fry were grown in 6m² (3m X 2m) fine-mesh hapas suspended in four 2000 m² ponds at a water depth of 0.5 m. In each pond, four hapas with different stocking densities were used: 5, 10, 15, and 20 fry m^{-2} which corresponds to 10, 20, 30 and 40 fry m^{-3}, respectively. Each day, dry chicken manure was spread over the surface of the ponds at a rate of 50 kg dry matter/ha/day. The chicken manure for this study was sourced from commercial layer and broiler farmers. Fry reared without supplementary feed (= NF) depended on naturally available food and phytoplankton induced by the manure (Bowen, 1981; Spataru et al., 1983). Those reared with supplementary feed (= SF) were fed on commercially available 40% protein feed (initially in form of paste balls, and later in form of pellets) in addition to the natural pond food. Each density and treatment combination was tested in duplicate. To avoid algal fouling, hapas were cleaned every two weeks and ponds stocked with fish of 0.68 g average weight at a density of 1 fish/m². Dissolved oxygen (DO), temperature and pH were measured daily (WTW® model multi 340i meter). Ammonia, nitrite and nitrate levels (HACH test kits) and turbidity (secchi disk visibility) were monitored every two weeks.

Average weight of fry at stocking was 0.015 g. Every two weeks, at least 20 fry/ hapa were collected, bulk-weighed and average wet weight recorded. The experiment lasted 53 days, allowing all fish to reach tagging size (>2 g). On the harvest day, fish were anaesthetized with MS222 (Tricaine methanesulphonate) and body weight measured for each fish.

2.2 Main Experiment

This experiment was conducted to determine genetic and environmental factors affecting early fry growth in hapa-in-pond nursing environments. We specifically considered the effects of pond, dietary treatment, and spatial arrangement of hapas on fry growth and estimated additive genetic and common environmental/ rearing hapa effects. Similar to the

pilot experiment, fry were reared on either naturally available pond food alone (NF) or with supplements of a 40% protein feed (SF).



Pond A			Pond B		
NS		NS	NS		NS
1		2	51		52
3		4	53		54
5		6	55		56
7		8	57		58
9		10	59		60
11		12	61		62
13		14	63		64
15		16	65		66
17		18	67		68
19		20	69		70
21		22	71		72
23		24	73		74
25		26	75		76
27		28	77		78
29		30	79		80
31		32	81		82
33		34	83		84
35		36	85		86
37		38	87		88
39		40	89		90
41		42	91		92
43		44	93		94
45		46	95		96
47		48	97		98
49		50	99		100
NS		NS	NS		NS

Figure 1. Schematic presentation of the hapa arrangement in Nile tilapia (*Oreochromis niloticus*) nursing ponds. Hapas at either end of columns indicated as NS were not stocked. They were used to ensure that each experimental hapa was enclosed on both sides.

2.2.1 Pond environments

Two 4500 m^2 ponds were used for this experiment. A total of 108 hapas (2m x 1m x 1 m) were installed at a water depth of 0.5 m— 54 hapas in each pond— making two parallel columns each of 27 rows of hapas (Figure 1). These columns were each two metres away from the pond bank and 67 meters apart from each other. Ponds were fertilized with chicken manure at the rate of 50 kg dry matter/ha/day. Temperature, dissolved oxygen and pH were measured twice a week inside each hapa at a depth of 30 cm and at four positions in each

pond outside the hapas. Sampling was done in the morning (6.00-7.00 h) and afternoon (15.00-16.00 h) with a portable DO meter (WTW® model multi 340i meter). Total ammonia, nitrate, and nitrite were monitored weekly in five random hapas per column.

2.2.2 Production and rearing of experimental fry

Grandparents of the experimental fish were produced in spring of 2000 from all possible diallele crosses between four local Egyptian strains (Rezk et al., 2002; 2004). Information on the production of parental generation is provided in more detail in Charo-Karisa et al., (2005). Twenty five full-sib families were produced by single pair mating of 25 dams and 25 sires in separate 6 m^2 hapas. Families were produced over a period of five weeks (23 August to 28 September 2003). At the end of spawning and nursing to swim-up, fry were removed from the hapas ready for stocking. One hundred and twenty swim-up fry were taken from each family and divided into four groups of 30 each. Fry groups were randomly stocked into the 2 x 1 x 1 m experimental hapas ensuring that each family was represented in each nursing pond and column. Fry were stocked at a density of 15 fry/ m^2 (= 30 fish m^{-3}). The remaining two hapas at either end of each column were not stocked to ensure that each experimental hapa was enclosed between two hapas.

Fry in one of the two columns in each pond had access to naturally available food only (NF). As in the pilot experiment, ponds were fertilized with dried chicken manure from commercial layer and broiler farms. In the other column, fry were fed twice daily (9.00 and 13.00 h) with commercial 40% protein formulated feed (SF), initially at 30% body weight. This was gradually reduced in subsequent weeks to 20% BW at the end of the experiment. Fish in this column also had access to the natural pond food. On day 14, 21, 28, and 35, fry were counted, bulk weighed and average weight recorded. To control hapa fouling, hapas were removed after bulk weighing of the fry, and cleaned by scrubbing the hapa sides before restocking with fry. The experiment was terminated on day 42 when all the fish were removed from the hapas, anaesthetized (MS222), and individual body weight and standard length measurements taken.

2.2.3 Parameters calculated

Survival rate for each hapa (S_t %) was calculated as $S_t = (N_t/N_0)$ X 100, where N_t is the number of fry at day t and N_0 is the number of fry at stocking. Fulton's condition factor was computed at the end of the experiment for each individual by the formula: $CF = 100W/L^3$ (Ricker, 1975), where W is body weight (g) and L is standard body length (cm). Specific growth rate of the fry was determined at harvest according to Cho and Kaushik, (1985): $SGR = (ln BW_f - ln BW_i) / t$, where BW_f is individual final wet body weight (in grams), BW_i is initial mean body weight for each family at stocking, and t is time interval between sampling in days. The coefficient of variation was calculated as standard deviation*100/ mean for body weight, standard length, specific growth rate, condition factor and survival.

2.3 Data analysis

Pilot experiment: the effect of stocking density and dietary treatment on mean fry body weight was evaluated using a linear model including the effects of stocking density and dietary treatment and their interaction as fixed effects and a random residual error effect. Differences among dietary treatment and density means were tested (P = 0.01) by least significant difference (LSD) using SAS (1989).

Main experiment: Due to heterogeneity of variances of fish among ponds, body weight data was log-transformed. Genetic and environmental effects and effect of spatial hapa arrangement on individual final body weight were analysed using the following model:

$$Y_{ijkl} = \mu + p_i + t_j + \beta_1 * log\,(INWT_{ijkl}) + \beta_2 * d_{ijkl} + h_k + u_l + e_{ijkl} \qquad \text{(Model 1)}$$

where Y_{ijkl} = logarithm of 42-day body weight of individual $ijkl$; μ is overall mean; p_i is fixed effect of pond (i = 1, 2); t_j is fixed effect of dietary treatment (j = 1, 2); β_1 is regression coefficient on logarithm of initial body weight; $log(INWT_{ijkl})$ is the co-variable logarithm of initial body weight (at stocking) of individual $ijkl$; β_2 is regression coefficient on number of fry in the hapa at the end of the experiment; d_{ijkl} is the co-variable number of fry in a hapa at the end of the experiment; u_l is a random additive genetic effect of individual l; h_k is a random effect of the kth hapa; and e_{ijkl} is a random residual effect associated with individual l.

In matrix notation Model 1 can be written as

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{u} + \mathbf{Z}_2\mathbf{h} + \mathbf{e}$$

with expectations

$$E(\mathbf{y}) = \mathbf{Xb},\ E(\mathbf{u}) = E(\mathbf{h}) = E(\mathbf{e}) = 0$$

and variance

$$v(\mathbf{y}) = \mathbf{Z}_1\mathbf{A}\mathbf{Z}_1' \sigma_u^2 + \mathbf{Z}_2\mathbf{G}\mathbf{Z}_2' \sigma_h^2 + \mathbf{I}\ \sigma_e^2$$

where, **X** is an incidence matrix for the fixed effects vector b (including co-variables); **u**, **h** and **e** are vectors with additive genetic, hapa and residual random effects, Z_1 and Z_2 are incidence matrices, **A** is the additive genetic relationship matrix, **G** is the correlation matrix of hapa effects and **I** is the identity matrix, σ_u^2 is the additive genetic variance, σ_h^2 is the hapa or the common environmental variance and σ_e^2 is the error variance. The pedigree consisted of 2379 fish, including parents and grandparents of the experimental fish.

The spatial autocorrelation of hapa effects was modeled using an autoregressive order one covariance structure which is separable in two dimensions (AR1x AR1). The G matrix was constructed by taking the direct product of the following matrices:

$$\begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \otimes \begin{bmatrix} 1 & \rho_r & \rho_r^2 & . & . & . \\ \rho_r & 1 & \rho_r & . & . & . \\ \rho_r^2 & \rho_r & 1 & . & . & . \\ . & . & . & . & . & . \\ . & . & . & . & 1 & \rho_r \\ . & . & . & . & \rho_r & 1 \end{bmatrix} \otimes \begin{bmatrix} 1 & \rho_c \\ \rho_c & 1 \end{bmatrix}$$

where ρ_r and ρ_c are the correlations between hapas across rows and columns, respectively. The second matrix is a 25 by 25 matrix reflecting the number of hapas that were stocked in a row in one pond. The spatial arrangement of the hapas (shown in Figure 1) resulted in the following G matrix:

Hapa number	Hapa number									
		1	2	3	4	5	6	.	51	52	.
	1	1	ρ_c	ρ_r	$\rho_r\rho_c$	ρ_r^2	$\rho_r^2\rho_c$	.	0	0	.
	2	ρ_c	1	$\rho_r\rho_c$	ρ_r	$\rho_r^2\rho_c$	ρ_r^2	.	0	0	.
	3	ρ_r	$\rho_r\rho_c$	1	ρ_c	ρ_r	$\rho_r\rho_c$	.	0	0	.
	4	$\rho_r\rho_c$	ρ_r	ρ_c	1	$\rho_r\rho_c$	ρ_r	.	0	0	.
	5	ρ_r^2	$\rho_r^2\rho_c$	ρ_r	$\rho_r\rho_c$	1	ρ_c	.	0	0	.
	6	$\rho_r^2\rho_c$	ρ_r^2	$\rho_r\rho_c$	ρ_r	ρ_c	1	.	0	0	.
								.			.
	51	0	0	0	0			.	1	ρ_c	.
	52	0	0	0	0			.	ρ_c	1	.
	.	.	.	.	.	.		.	.	.	.

The autoregressive order one covariance structure assumes that covariance between hapas decreases with distance. For example, the autocorrelation between a hapa and its immediate neighbour in a row was ρ_r and with its next neighbour was ρ^2_r. Hapas in different ponds were assumed to have no covariance between them. Solutions were obtained using ASREML program (Gilmour et al., 2002). Significance of including spatial autocorrelation in the model was evaluated using likelihood ratio test between Model 1 and a nested model in which the covariance structure of hapa arrangement was absent i.e. hapas were considered independent units.

Heritability (h^2) and common environmental/hapa effects (c^2) for final (day 42) body weights were obtained for the complete data set using Model 1. We computed h^2 as the ratio between the additive genetic variance component and total phenotypic variance, and c^2 effect as the ratio between hapa variance component and the total phenotypic variance. A bivariate setting of Model 1 was used to estimate h^2, c^2 and genetic correlation (r_g) between body weight measured in pond A and pond B. In the bivariate analysis, we considered final fry body weight in pond A and in pond B as separate traits. Except for pond effect, the bivariate model included all the other effects in Model 1. The r_g estimated between the two traits was used to evaluate the presence of genotype by environment interaction.

The effects of water quality (morning and afternoon dissolved oxygen, temperature, and pH measured in each hapa), and pond, treatment and week of sampling on body weight were determined by the GLM procedure of SAS (1989) with the following model:

$$Y_{ijkl} = \mu + p_i + t_j + w_k + \beta_1 * d_{ijkl} + \beta_2 * DOM_{ijkl} + \beta_3 * DOA_{ijkl} + \beta_4 * TM_{ijkl} + \beta_5 * TA_{ijkl} + \beta_6 * PHM_{ijkl} + \beta_7 * PHA_{ijkl} + e_{ijkl} \quad \text{(Model 2)}$$

where Y_{ijkl} is logarithm of mean body weight of fish in the *l*th hapa at each sampling (i.e. each week); u is overall mean; p_i is fixed effect of pond (i = 1, 2); t_j is fixed effect of dietary treatment (j = 1, 2); w_k is fixed effect of the *k*th week of sampling (k = 1, 2, 3,…6); β_1 is the regression coefficient on number of surviving fish; d_{ijkl} is the covariable number of surviving fish; β_2 is the regression coefficient on morning dissolved oxygen (DOM); DOM is the covariable morning DO in each week; β_3 is the regression coefficient on afternoon dissolved oxygen (DOA); DOA is the covariable afternoon DO in each week; β_4 is the regression coefficient on morning temperature (TM); TM is the covariable morning temperature in each week; β_5 is the regression coefficient on afternoon temperature (TA); TA is the covariable afternoon temperature in each week; β_6 is the regression coefficient on morning pH (PHM); PHM is the covariable morning pH in each week; β_7 is the regression coefficient on afternoon pH (PHA); PHA is the covariable afternoon pH in each week; and e_{ijkl} is a random residual effect associated with the *l*th hapa. DOM, DOA, TM, TA, PHM and PHA in the model were averages of the two weekly measurements in each hapa.

We estimated the Pearson correlation coefficients between final body weight and the hapa effects (i.e. the hapa solutions from Model 1) and average DOM, DOA, TM, TA, PHM and PHA readings in each hapa.

3. Results

3.1 Pilot experiment

Growth curves for both natural fed (NF) and supplementary fed (SF) fry in the preliminary trial are shown in Figure 2. In the first 26 days, growth at 5 fry/m² density was better in the NF than in the SF treatment. By day 40, the NF growth curves started to level-off and growth in the 5 fry/m² NF hapas decreased below that of the SF treatment. The same trend was observed in the 15 and 20 fry/m^2 NF hapas. The growth trend in the 10 fry/m^2 NF was similar to that in 15 and 20 fry/m^2 SF treatments. By day 53, dietary treatment and stocking density and their interaction had significant effect on growth rate and final fish body weight

(P<0.001). Mean final body weights at 15 fry/m² density was 4.1 g in the NF treatment 7.3 g in the SF treatment.

3.2 Main experiment

3.2.1 Descriptive statistics

Final fry body weights in the main experiment are presented in Table 1; separately for each pond. By day 42, mean fry body weight in pond A was 1.75 g in the SF treatment and 1.68 g in the NF treatment. In pond B, mean body weight was 0.44 g in the SF and 0.37 g in the NF treatment. Overall, mean 42-day body weight was 1.7 g in pond A and 0.4 g in pond B. Survival in the two ponds was generally high in both treatments (range, 86.9 to 89.3%). Coefficients of variation for growth parameters and survival differed slightly within the two treatments in each pond. The coefficient of variation for growth was higher in pond B than in pond A.

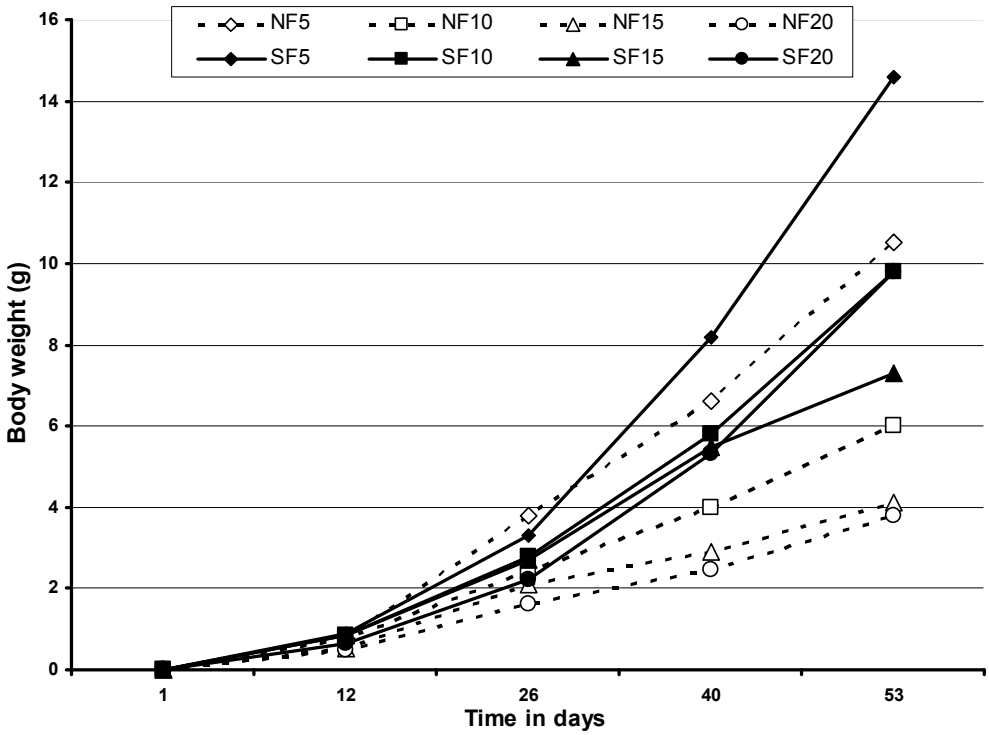


Figure 2. Growth curves of Nile tilapia (*Oreochromis niloticus*) fry grown with (SF) or without supplementary feeds (NF) at different densities. Numbers refer to the fry stocking densities per square meter.

3.2.2 Fixed and covariable effects

Results from the complete data set indicate that the effects of pond and initial body weight on final body weight were significant (P = 0.01; with F-statistics in the magnitude of 426 and 12.9 respectively). Neither fish density at the end of experiment, nor the dietary treatment effects or their interactions was significant.

Table 1. Final body measurements, overall growth rate, condition factor, and overall survival of Nile tilapia fry reared in hapas suspended in two nursing ponds (A and B). Fry were from the same families. Fry in supplementary fed (SF) treatment received 40% protein supplements while those in the natural-fed (NF) treatment fed on natural pond food only.

	Supplementary fed		Natural-fed	
Parameter	Mean	CV (%)	Mean	CV (%)
Pond A				
Body weight (g)	1.75	41.0	1.68	36.9
Standard length (mm)	36.32	13.0	36.19	12.4
Specific growth rate	8.94	15.4	8.99	16.1
Condition factor	3.52	18.8	3.42	12.9
Survival (%)	87.7	23.3	87.7	20.5
n	573		572	
Pond B				
Body weight (g)	0.44	34.1	0.37	40.5
Standard length (mm)	23.64	11.2	22.90	9.9
Specific growth rate	5.64	31.4	5.34	31.5
Condition factor	3.22	18.3	2.99	19.1
Survival (%)	89.3	17.4	86.9	23.0
n	584		565	

3.2.3 Heritability, common environmental effects, genetic correlation, and spatial autocorrelation

The h^2 obtained with the univariate analysis of the whole data set was 0.01±0.06 with a c^2 of 0.36±0.05 (Table 2). Spatial autocorrelations were 0.29 across rows and 0.22 across columns. The likelihood ratio test of Model 1 before and after correction for spatial autocorrelation revealed that the spatial autocorrelation effect was significant ($\chi_2^2 = 7.6$; $P = 0.02$).

In the bivariate model, the h^2 estimated in Pond A was higher (0.59±0.19) than in pond B (0.05±0.11). On the other hand, c^2 effect was lower in pond A (0.14±0.06) than in pond B (0.29±0.07). The r_g for the two traits (body weight in pond A and body weight in pond B) was -0.27±0.69. In pond B, the spatial autocorrelation was 0.33 across rows and 0.26 across columns, while in pond A they were 0.16 and -0.06, respectively.

Table 2. Heritability (h^2), common environmental effect (c^2), total phenotypic variance (V_P), genetic correlation (r_g) estimates for log transformed body weights in pond A and B, and the spatial autocorrelations across rows (ρ_r) and columns (ρ_c) within ponds. Body weight in each pond was considered a distinct trait and was used for estimation of r_g.

Trait	h^2	c^2	V_P	r_g	ρ_r	ρ_c
Univariate						
BW in both ponds	0.01 (0.06)	0.36 (0.05)	0.15	-	0.29	0.22
Bivariate						
BW pond A	0.59 (0.19)	0.14 (0.06)	0.16	-0.27 (0.69)	0.16	-0.06
BW pond B	0.05 (0.11)	0.29 (0.07)	0.15		0.26	0.33

3.2.4 Effect of water quality on body weight

We analyzed the effect of temperature, pH, and dissolved oxygen on final body weight in both ponds. Morning and afternoon temperatures ranged from 20.2 - 24.4˚C and from 23.3 - 30.3 ˚C respectively, while morning and afternoon pH were between 8 -10.2 and 7.9 -11.3. Morning DO readings ranged from 1.5-8.3 mg l^{-1} in pond A and from 0.6 - 3.7 mg l^{-1} in pond B. Afternoon DO was between 6.0-20.0 mg l^{-1} in pond A and 5.9-20.4 mg l^{-1} in pond B.

The effect of pond, treatment, week of sampling and water quality parameters (Model 2) on fish body weight is shown in Table 3. Body weight was significantly affected by pond, week of sampling, fish survival at the end of each week, morning and afternoon DO, and afternoon pH. Dietary treatment and temperature did not significantly affect body weight. The first order interaction between week and treatment was not significant.

Table 3. Marginal (Type III) sum of squares of the effects water quality, pond, fish survival, sampling week and dietary treatment effects on body weight of Nile tilapia fry reared in hapa-in pond system.

Source	DF	Type III SS	F value	*P*-value
Pond	1	31.17	167.08	<.001
Week	5	544.91	584.13	<.001
Dietary Treatment	1	0.19	1.03	0.31
Fish survival	1	1.17	6.29	0.01
Morning DO (mg l^{-1})	1	1.11	5.95	0.02
Afternoon DO (mg l^{-1})	1	1.00	5.38	0.02
Morning Temperature (˚C)	1	0.04	0.23	0.63
Afternoon Temperature (˚C)	1	0.16	0.88	0.35
Morning pH	1	0.13	0.69	0.41
Afternoon pH	1	0.85	4.57	0.03
Error	549	102.4		
R^2	0.88			

The correlations between hapa effects (hapa solutions from Model 1) and body weight as well as with water quality parameters were low and non-significant. On the other hand, we found highly significant correlations between mean body weight per hapa and morning and afternoon DO per hapa (r = 0.87 and 0.51 respectively), and morning and afternoon pH (r = 0.85 and 0.67 respectively). The correlation between body weight and morning temperature was negative (r = -0.58).

4. Discussion

In this study, we found differences in body weight, and heritability (h^2) estimates and common environmental/ hapa (c^2) effects among ponds. However, fry body weights and estimates of heritability and common environmental/ hapa effects did not differ among dietary treatments (results not shown). The difference in body weight in different ponds is consistent with earlier findings and is a major problem with pond aquaculture experiments (Smart et al., 1997; Riley and Edwards, 1998) because the differences may persist even though ponds are treated similarly (Riley and Edwards, 1998). Smart et al. (1997) suggested that under certain circumstances, a crossover design may be used to improve precision of pond aquaculture experimentation. In the present experiment, a cross-over design was implemented in which the same families were reared in two treatments applied simultaneously in two ponds.

Higher h^2 estimates were found in the better performing pond (i.e. pond A). The h^2 estimates from the complete data set and the poorly performing pond (pond B) were consistent with earlier estimates by Tave and Smitherman (1980), who reported h^2 estimates of 0.04 for both 45 and 90-day body weights for *O. niloticus* in fertilized pools and received supplementary feed. The lower heritability in Tave and Smitherman (1980) may be attributed to a small founder population which also experienced severe reductions in effective breeding numbers (Teichert-Coddington and Smitherman, 1988). The coefficients of variation for body weight in the present study were 4-5 times higher than those in Tave and Smitherman (1980). This level of variation may be because the founder population in this study was much larger and was a synthetic stock from four local Egyptian strains (Rezk et al., 2002; 2004). The high heritability estimates in pond A and the higher variation in body weight in this study indicate that selection for early growth in Nile tilapia is feasible.

We found large differences in h^2 estimates in the two nursing ponds, even though the same full-sib families and management levels were used in both ponds. Similar findings have been reported for slaughter weight of rainbow trout reared in three similar farms in Norway (Sylven et al., 1991), and for sexual maturity in cage-cultured Atlantic salmon (Wild et al., 1994). Similarly, Garant et al. (2003) found lower h^2 estimates for growth of salmon (*Salmo salar*) in natural habitats which had lower food resource than in habitats with optimal resource availability. In contrast to the present study, Sylven et al. (1991) found that farms with the highest performance also showed lowest heritability, which was attributed to increase in residual variances while the sire variances were of the same magnitude across environments.

Environments can be categorized as bad or good depending on their effect on the target trait, with bad being the case where expression of the trait is suppressed and good where it is enhanced (Falconer, 1990). Differences in h^2 estimates in different environments can occur due to a decrease in the additive genetic variance component in the bad environment or due to a different set of genes acting in the good and bad environment (Falconer, 1989). A decrease in additive genetic variance component in the bad environment is a result of low expression of genes affecting the trait (Falconer, 1989). The large difference in h^2 estimates and the low r_g estimates in the two nursing ponds, suggests differences in expression of additive genes responsible for growth in these environments.

The estimated genetic correlation (r_g) for body weight among ponds in this study was well below unity and negative, suggesting the presence of substantial genotype by environment (GXE) interactions. Ignoring GXE interactions may bias h^2 estimates (Mitchell-Olds and Rutledge, 1986; Via, 1984) and could have led to the lower heritability estimates in the complete data set. Low r_g between traits expressed in different environments is an indication that the traits are not influenced by the same genes (Falconer, 1989). However, due to the high standard errors in the r_g estimate we cannot conclude whether this was true in the present study. Studies of genotype by environment interactions in Nile tilapia have not been conclusive, and are limited to strain by environment interaction. Macaranas et al. (1997), evaluated four Nile tilapia strains under two pond culture systems (integrated and non-integrated). Bentsen et al. (1998) evaluated eight Nile tilapia strains under eight different

farm environments. Both studies indicated that there were no GXE interactions. However, Romana-Eguia and Doyle (1992) found strong GXE interactions for tolerance to poor nutrition in juveniles of three Nile tilapia strains. Although the r_g estimates in this study are not conclusive, the mean performance of full-sib families (Figure 3) indicates presence of GXE interactions for growth in ponds.

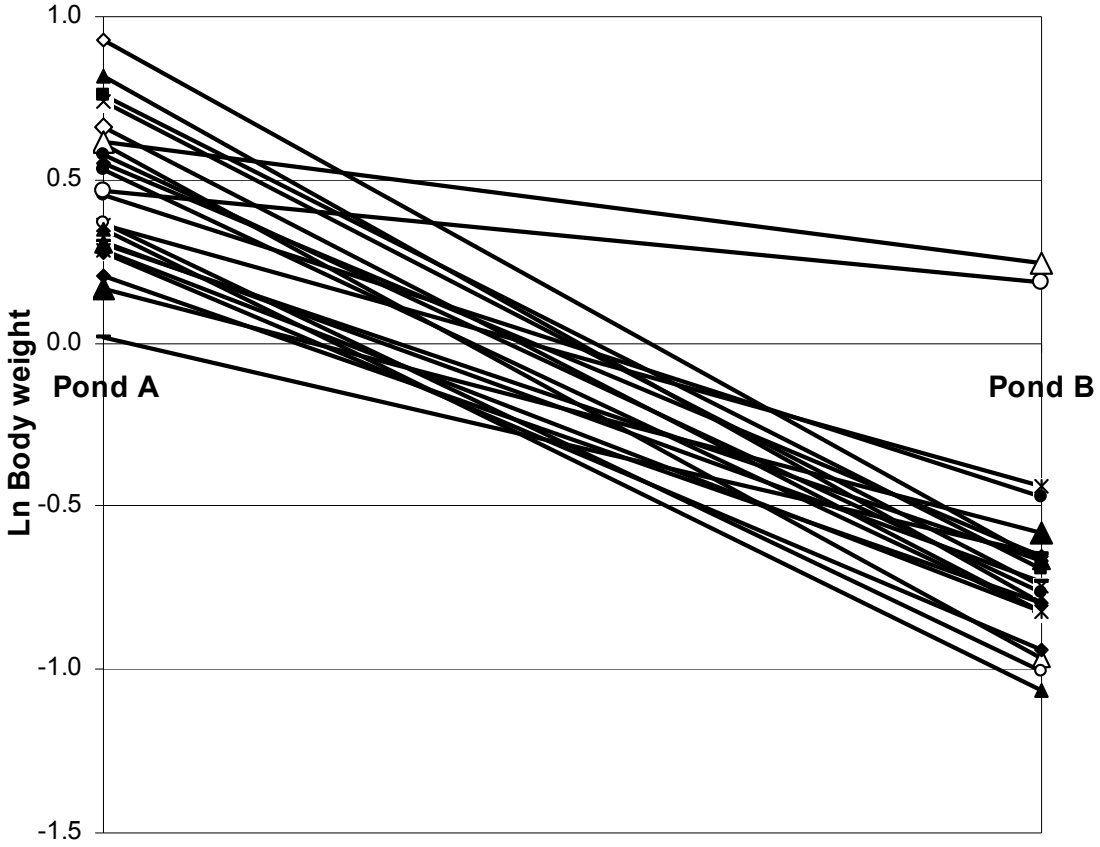


Figure 3. Final mean body weight of full-sib families of Nile tilapia (*Oreochromis niloticus*) reared in two different pond environments. Mean body weights were obtained through averaging of individual (Ln transformed) body weights from each full-sib family in each pond.

We found higher heritability and lower common environmental/ hapa effects in pond A, while in pond B we found lower heritability and higher common environmental/ hapa effects (c^2). The c^2 effect in this study included dam influences (including egg size, incubation and pre-swim-up rearing), a quarter of the non-additve genetic effects, as well as the effect of hapas during the growth experiment. In the present study, low h^2 and higher c^2 effects occurred in the "bad" pond. Merila (1997) indicated that this is a common phenomenon in

poor bird and plant environments. Common environmental effects are important because they can complicate estimation of genetic parameters (Ponzoni et al., 2005). Furthermore, although common environmental effects generally decrease with time, they can be a problem during grow-out stages. Rutten et al. (2005) showed that they can persist even when the fish are much older. Therefore, c^2 effects should be minimized during early growth. Because a part of the c^2 effect consists of hapa effects, c^2 may be decreased by making the environment more uniform through management of the environmental factors that increase hapa effects.

In this study, we found significant positive spatial autocorrelations, indicating that hapas were not independent of each other. If hapas were completely independent, there would have been no spatial autocorrelations. This means that in pond B neighboring hapas resembled each other more than in pond A. In field experiments, spatial autocorrelations may arise because of the action of the spatial structure of the environment upon the response variable (Legendre et al., 2004). Since families were randomized over the ponds, the observed spatial autocorrelations indicate some variation in spatial patterning of environmental variables that influence growth. The differences in spatial autocorrelations and in levels of dissolved oxygen among ponds may have been due to variation in water circulation in the ponds. In theory, estimates of heritability become reduced if environments are heterogeneous because of increase in the environmental component of variance (Mitchell-Olds and Rutledge, 1986; Simons and Roff, 1994). This may explain the lower heritability in the complete data set compared to individual pond heritabilities, and the lower h^2 estimates in pond B than in pond A.

Fish growth is affected by severable variables, key among them being fish size, DO, and water temperature, photoperiod, and food availability (Yi, 1999; and references therein). Among all water quality parameters measured, only morning and afternoon DO, and afternoon pH affected fish body weight (Table 3). Although one excellent attribute of tilapia is their tolerance to low DO concentrations, extended periods of hypoxia may reduce growth (Chervinski, 1982) and cause mortality (Coche, 1982). However, we did not find any differences in survival between ponds or treatments in this study. Because feed conversion in fish directly depends on oxygen availability, food and oxygen are two interrelated factors that act to limit growth (Adelman and Smith, 1970; Brett, 1979; Buentello et al., 2000). This

indicates that the differences of body weight between ponds had to do with the amount of DO available in the hapas and ponds. We twice weekly measured the amount of morning and afternoon DOs directly in each hapa. Although we did not find significant correlations directly between hapa effects (i.e. the hapa solutions from Model 1) and DO in the hapas, we found highly significant correlations between DO and mean fry body weights. In ponds, DO is mostly influenced by aeration type and the nature of pond bottoms (Milstein et al., 2001). The amount of DO in hapas largely reflected the levels of DO in the pond. The resultant high spatial heterogeneity and low growth in pond B is likely to be due to differences in pond bottoms, since pond A had recently been excavated of pond mud.

In the present study, we found some evidence that DO might be a major factor causing GXE. However, Wild et al. (1994) urged for caution in attributing GXE to specific environmental factors because all variables at any particular site add up to the total "net effect" on a trait. Nevertheless, the differences in performance of the same families observed in the two ponds in the present study suggest that some tilapia genotypes may tolerate stressful conditions such as low dissolved oxygen better than others. The observed GXE interaction, although inconclusive, hints to the potential for selecting for tilapia strains that are tolerant to low dissolved oxygen. Macaranas et al. (1997) suggested that GXE interactions are best investigated in market-sized fish. However, juvenile fish may as well be considered to be close to marketing when they are sold as fish seed. The differences in growth performance of genetically identical fry may portend economic losses in the poorer nursing environments. This is of great relevance for poor farmers who cannot improve aeration in their nursing ponds. Furthermore, it is not known how poorer growth performance during the nursing stage affects subsequent grow-out performance.

5. Acknowledgements

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Chapter 3

Heritability of cold tolerance in Nile tilapia (*Oreochromis niloticus*) juveniles

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Abstract

The inability of tilapia to tolerate low temperatures is of major economic concern as it reduces their growing season and leads to over winter mortality. In this study, cold tolerance of juvenile Nile tilapia, *Oreochromis niloticus* was investigated and heritability estimates obtained. A total of 80 maternal full-sib families were produced by mating each sire with two dams. Fry were grown in hapas suspended in earthen ponds fertilized with chicken manure, and were 41-91 days post-hatch at the start of the experiment (mean standard length 50.6 mm; mean body weight 5.1 g). Fry were tagged and exposed to low temperature in an indoor facility. Temperature was lowered from 16 °C to 11°C in 48 hours and from 11 °C to 8 °C at the rate of 1°C/day. Cold tolerance was expressed as Temperature at Death (TAD) and Cooling Degree hours (CDH). Fish mortality started at 13.6 ºC and total mortality occurred at 8.6 ºC. Mean TAD and CDH were 10.1 ºC and 298.07 respectively. Fish body weight (BW) had a highly significant effect on cold tolerance (P <0.0001). Smaller fish (<5g) were more susceptible to lower temperature than larger fish. The heritability of cold tolerance was 0.08±0.17 for CDH and 0.09±0.19 for TAD, estimated with an animal model. There was a considerable common environmental/full-sib effect for this trait (0.33±0.10 for CDH and 0.27±0.09 for TAD). These values indicate that estimation of genetic parameters for cold tolerance in tilapia should include both direct additive and common environmental effects. Based on the results of this study we conclude that the most appropriate way of enhancing cold tolerance of tilapia juveniles is by husbandry practices that increase pre-winter body weights.

Keywords: Cold tolerance; Nile tilapia; *Oreochromis niloticus*; heritability; selection

1. Introduction

Tilapias are among the most important warm water fishes used for aquaculture production. They originate from the tropical and subtropical parts of Africa (Fryer and Iles, 1972) but are now farmed throughout the world. In temperate and some sub-tropical regions, their culture is highly affected by sensitivity to low ambient temperatures leading to poor growth and mass mortality during over-wintering (Chervinski and Lahav, 1976; Tave et al., 1990). This restricts the grow-out period in these regions to between three and seven months (Hofer and Watts, 2002). To maximise the grow-out season, fingerlings are usually produced indoor during colder months and stocked during warmer summer periods. The optimal temperature for

growth of most tilapiine species is between 25- 28 °C. Reproduction stops at 22 °C and feeding below 20 °C (Wohlfarth and Hulata, 1983). Tilapia cannot survive temperatures less than 10-12 °C for more than a few days (Chervinski, 1982).

A few studies have been carried out on the genetic basis of cold tolerance in tilapia. Wohlfarth et al. (1983) and Cnaani et al. (2000) studying some tilapia species and their hybrids found that a large component of the trait's variance was a result of dominance effects. But in Nile tilapia, *Oreochromis niloticus,* Tave et al. (1989, 1990) and Behrends et al. (1990) suggested that cold tolerance is controlled by additive genes. However, the only estimate of heritability for cold tolerance in *O. niloticus* reported so far was close to zero (-0.05; Behrends et al., 1996).

A collaborative project aimed at selecting for fast growth of *O. niloticus* in ponds is currently being carried out by Wageningen University, The Netherlands, and The WorldFish Centre (formerly ICLARM) Regional Centre for Africa and West Asia, Abbassa, Egypt. Abbassa is located east of the Nile Delta, which experiences cold spells during winter. Knowledge and improvement of the temperature tolerance of fingerlings could help extend the grow-out period and reduce economic losses in this region. The main objective of this study was therefore to estimate the heritability of cold tolerance in juveniles of *O. niloticus*.

2. Materials and methods

2.1. Historical background and fish production

All experimental procedures were conducted at The World Fish Regional Centre Experimental Station at Abbassa. Grandparents of the experimental fish were produced in spring of 2000 from all possible diallel crosses between four local Egyptian strains (Rezk et al., 2002; 2004). 108 brooders from the grandparental stock were randomly mated in 2002 to produce the parental fish. Grand-sires were each mated to either one or two females. The experimental fish were produced in a full-sib/half-sib mating design in which each sire was mated to two dams and each dam mated to only one sire. A total of 80 full-sib families were produced from 43 sires and 80 dams. These consisted of 37 paternal half-sib families, six sires having been mated to one dam each. The pedigree of all the individuals was known and was used to construct a relationship matrix. The number of individuals used in the pedigree and the fish production scheme is shown in Figure 1.

Fry were produced over a period of 60 days. During this period 60 fry from each full sib family were reared in 80 separate 2 x 3 m hapas until the last family reached tagging size (mean of 2g). Hapas were set in two 1000 m^2 earthen ponds that received a daily application of chicken manure at the rate of 50kg/ha. No supplemental feeds were given throughout the growth period. Fry were 41-91 days old at the beginning of the cold tolerance challenge.

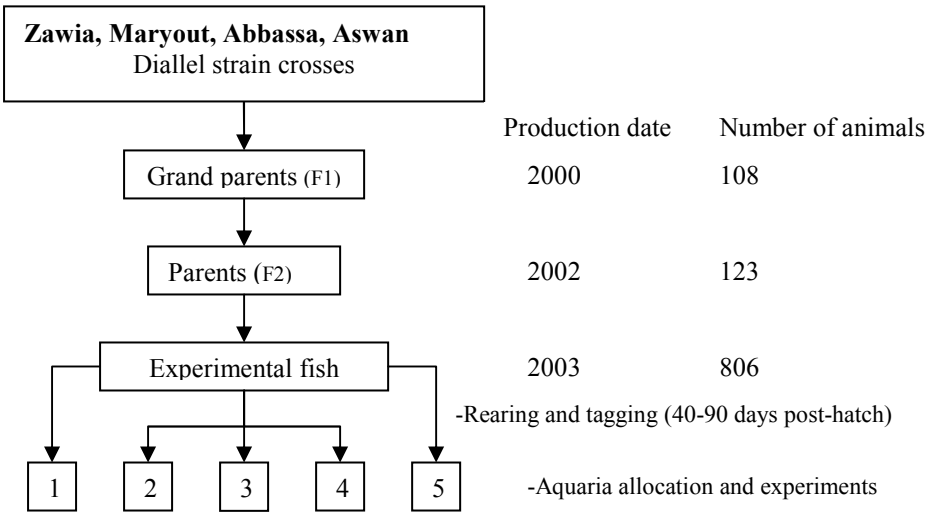

Figure 1. Schematic presentation of production of experimental fish from four local *Oreochromis niloticus* strains from different parts of Egypt.

2.2. Cold tolerance challenge

From each full-sib family, 12 randomly chosen individual fry were tagged with Floy tags between the dorsal fin and lateral line. Tagged fish were returned to hapas and allowed to recover from tag stress for four days. Individual body weights and standard lengths were recorded and then the fish (in groups of 5 families each in separate 1 x 1 m netting compartments) were kept for one day in ceramic tanks at ambient temperature (20 ºC). On the next day, ten healthy individuals from each family were randomly assigned to any two of five 450 l glass aquaria placed in a 1.8 by 3 m cold room served by a thermostatically controlled chilling unit. The assignment was such that each aquarium in the end contained 160 fish, from 32 half-sib families. Each aquarium was constantly aerated using three air-stones connected to an air-pump. The temperature of the aquarium water was adjusted to the desired level by adjusting the compressor settings of the chiller. Fish were acclimatized to aquarium conditions for 48 hours with water temperature maintained at 20 ºC.

Aquarium water temperature was monitored each hour from the beginning to the end of the experiment. Apart from the hourly temperature measurements, dissolved oxygen (DO), pH, total ammonia, nitrate and nitrite, were measured once a day, using a WTW® multi 340i meter and HACH kits. Dissolved oxygen ranged between 6.1-10.3 mg/l; pH, 7.9-8.3; ammonia, 0.01-0.1 mg/l; nitrate, 0.5-2 mg/l; and nitrite, 0.01-0.02 mg/l. Aquaria were cleaned twice daily by suction to remove faeces. Water that was removed during aquarium cleaning was replaced with clean water pre-cooled to the same temperature with ice cubes.

Fish were not fed during the experiment. Temperature was first lowered to 16 ºC within 48 hours, and then to 11 ºC within the next 48 hours. From 11°C to the end of the experiment, water temperature was reduced at a rate of 1°C per day. Aquaria were observed once each hour for any fish mortality. Death was defined as the point at which fish lost balance, fell on their side and ceased fin, body and opercula movements and lost response to external stimuli. Throughout the experiment, dead fish were removed from the tanks at the end of each hour with a scoop net, and their tag and aquarium numbers recorded. Mortality was recorded hourly for each fish, which enabled us to quantify cooling degree hours (CDH) in line with Cnaani et al. (2000): the sum of hours the fish survived multiplied by the difference between the hourly and initial temperature for each fish. As in earlier studies (Behrends et al., 1996; Cnaani et al., 2000; 2003), the initial temperature for calculation of cooling degree hours (CDH) was set at 16 ºC. Temperature at death (TAD), recorded hourly, was used in this study as a second measure of cold tolerance.

2.3. Data analysis

Initially, the effect of age, aquarium, sire, dam and size (body weight and standard length) on cold tolerance was tested using the GLM procedure of SAS (1989). Due to the high correlation between body weight and length, standard length was not included in the final model. The following model was eventually fitted using Proc GLM in SAS:

$$Y_{ijkl} = \mu + a_i + \beta_1 * AGE_{ijkl} + \beta_2 * ln(w_{ijkl}) + s_j + d_k(s_j) + e_{ijkl} \qquad \text{(Model 1)}$$

Where Y_{ijkl} = cooling degree hours or temperature at death for an individual, μ = overall mean, a_i = fixed effect of aquarium (i= 1, 2, 3, 4, 5), β_1 = regression coefficient of age; AGE_{ijkl} = a co-

variable of age of the *l*th individual; β_2 = regression coefficient of natural logarithm of body weight, $ln(w_{ijkl})$ = a co-variable of natural logarithm of body weight of an individual, s_j= effect of the *j*th sire, $d_k(s_j)$ = effect of the *k*th dam nested within the *j*th sire, and e_{ijkl} = random residual effect associated with an individual.

Estimation of heritability was performed using the ASREML package (Gilmour et al., 2002). The individual (animal) model was used to estimate genetic parameters using all the experimental fish and their relatives. A pedigree file with the relationship between the experimental fish, their parents and grandparents was included in the analysis. The fitted model was

$$Y_{ijk} = \mu + a_i + \beta * AGE_{ijk} + d_j + u_k + e_{ijk} \qquad \text{(Model 2)}$$

where Y_{ijk} is CDH, TAD or natural logarithm of body weight (LnBW); μ is the overall mean; a_i is a fixed effect of aquarium (i = 1, 2, 3, 4, 5); β is a regression coefficient of age; AGE_{ijk} is the effect of age on observation ijk; u_k is a random additive genetic effect of the *k*th individual; d_j is a random effect for full-sib group j, and e_{ijk} is a random residual effect associated with observation ijk. For the analysis of cold tolerance traits (CHD or TAD), Model 2 was then modified by including the natural logarithm of body weight as a co-variable to correct for weight differences. The heritability was calculated as the ratio between animal genetic variance and the total phenotypic variance. The common environmental/ full-sib effect was computed as the ratio between the dam variance and the total phenotypic variance. This may also contain a quarter of the non-additive genetic effects, and includes environmental effects caused by separate rearing of full-sib families until tagging. Phenotypic and genetic correlations between CDH and TAD and natural logarithm of body weight were estimated in bivariate animal model analyses using the variance components from the univariate analyses of CDH and TAD as starting values. The model included both the additive genetic and common environmental/ full-sib effects together with age and aquaria effects (Model 2).

3. Results

Cold tolerance values and fish size measurements are shown in Table 1. Mean TAD and CDH were 10.1 ºC and 298.07 respectively. There was a much higher phenotypic variation in cold

tolerance when expressed as CDH (CV= 22.8) than TAD (CV=5.5). Mean standard length and body weight were 50.61 mm and 5.06 g respectively. Smaller fish tended to have lower CDH values. Mortality started gradually at 13.6 ºC but increased dramatically between 10.5-10.1 ºC. Complete mortality occurred at 8.6 ºC (Figure 2).

Table 1. Overall means and standard deviations of body weight, standard length, age and cold tolerance responses of *Oreochromis niloticus* juveniles exposed to experimentally lowered temperatures

Trait	Mean	Standard deviation	Minimum	Maximum
Body weight (g)	5.10	2.35	1.0	20.6
Standard length (mm)	50.61	7.60	29.6	78.0
Age (days post-hatch)	79.00	8.62	41.0	91.0
Temperature at death (ºC)	10.10	0.56	8.6	13.6
Cooling degree hours	298.07	67.86	6.4	440.3

The GLM analysis (Model 1) revealed significant effects ($P < 0.001$) of aquarium, family groups and body weight on cold tolerance (Table 2). When body weight was included in the model, the effect of standard length was rendered insignificant. In the presence of body weight age did not have a significant effect. Model 1 explained approximately 55% of the observed variance of cold tolerance when the trait was expressed as CDH and approximately 50% when expressed as TAD. One-way analysis of variance of CDH values revealed differences due to sires ($P= 0.0001$) and due to dams ($P= 0.0001$), and among aquaria ($P = 0.0001$). However, aquaria were not different with respect to temperature or any of the water quality parameters measured.

Table 2. Marginal (Type III) mean square values of the effects in Model 1 on cooling degree hours (CDH) and temperature at death (TAD). All effects shown were significant ($P < 0.001$)

Source	df	Mean square values	
		CDH	TAD
LnWT	1	219184.1	10.37
Aquarium	4	25403.7	1.64
Sire	36	8259.2	0.57
Dam(sire)	37	7208.9	0.54
Error	633	2304.1	0.17
R^2		0.55	0.49

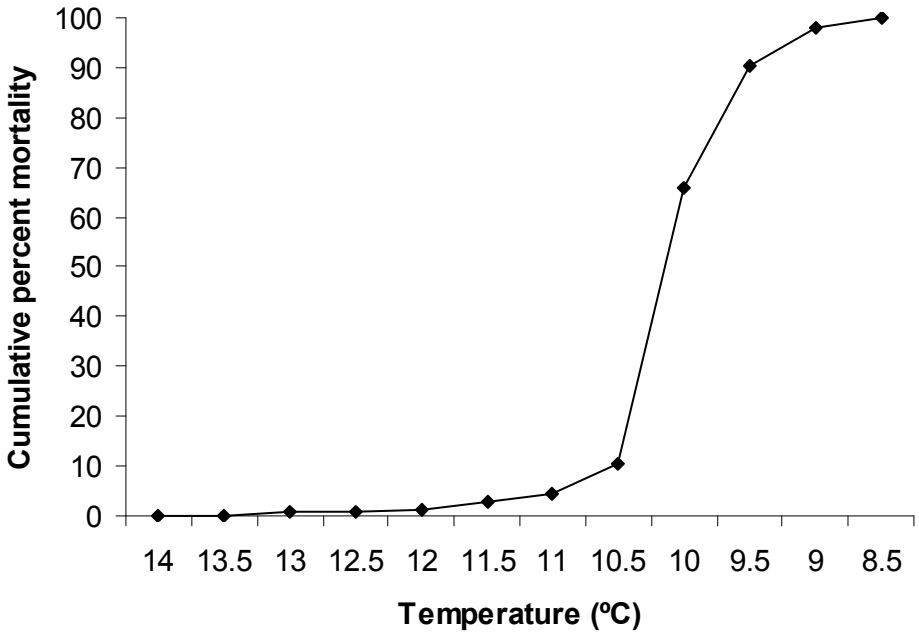

Figure 2. Cumulative mortality of *Oreochromis niloticus* juveniles under different temperatures during cold tolerance challenge

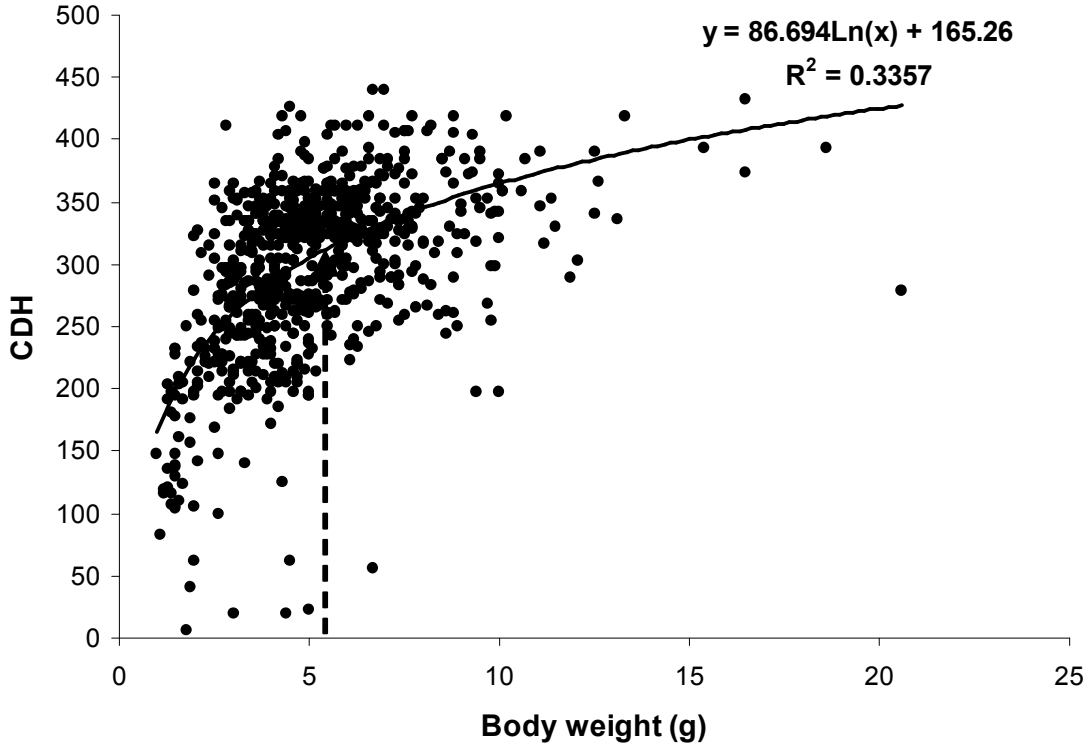

Figure 3. The relationship between cooling degree hours (CDH) and body weight in *Oreochromis niloticus* juveniles

The relation between cooling degree hours and body weight was not linear but could be described by a logarithmic relationship (Figure 3). The variable body weight was hence replaced with its natural logarithm in the analysis. With increased size, the relationship between CDH and body weight becomes approximately linear.

Estimates of heritability (h^2) for CDH and TAD and their standard errors are given in Table 3. The heritability of cold tolerance traits without correction for body weight was 0.08 for CDH and 0.09 for TAD. The proportion of the variation due to full-sib family effects were 0.32 and 0.27 for CDH and TAD respectively indicating significant common environmental/full-sib effects (c^2) in both cases. The full sib family effects might be due to maternal effects or common hapa effects caused by separate rearing of full-sib families before tagging. When CDH and TAD were corrected for body weight, the h^2 estimates were reduced to 0.05 and 0.06 respectively. The c^2 estimates were also reduced (by more than half) to 0.14 and 0.11 for CDH and TAD respectively. This indicates a substantial effect of body weight on especially the common environment effects of cold tolerance traits. The heritability for LnBW was 0.1 with c^2 of 0.6.

Table 3. Heritability estimates (h^2) and standard errors (*SE*) of cold tolerance traits: cooling degree hours (CDH) and temperature at death (TAD) adjusted and unadjusted for body weight and natural log of body weight (LnBW) according to Model 2. c^2 are common environmental/ full-sib effects other than additive genetic effects and σ^2_P, total phenotypic variance.

	Parameter		
Trait	$h^2 \pm SE$	$c^2 \pm SE$	σ^2_P
Cooling degree days (CDH)	0.08 ± 0.19	0.33 ± 0.10	4530
adjusted for LnBW	0.05 ± 0.11	0.14 ± 0.06	2976
Temperature at death (TAD)	0.09 ± 0.17	0.27 ± 0.09	0.31
adjusted for LnBW	0.06 ± 0.17	0.06 ± 0.10	0.23
LnBW	0.10 ± 0.30	0.61 ± 0.14	0.20

There was a negative phenotypic correlation between CDH and TAD values (-0.88 ± 0.019) indicating a strong relationship between the two descriptors of cold tolerance. The genetic correlation between CDH and TAD was (-0.99 ± 0.06). However, CDH was more precise than TAD in distinguishing between fish that died at the same temperature but at different times (Figure 4). The phenotypic correlation between LnBW and cold tolerance was not very strong (0.33 ± 0.17 and -0.26 ± 0.18 for CDH and TAD, respectively). The genetic correlation

between cold tolerance and body weight was stronger (0.72 ±0.81 with CDH; -0.68 ± 0.84 with TAD). However, these genetic correlations could not be estimated very accurately as is reflected by the very high standard errors.

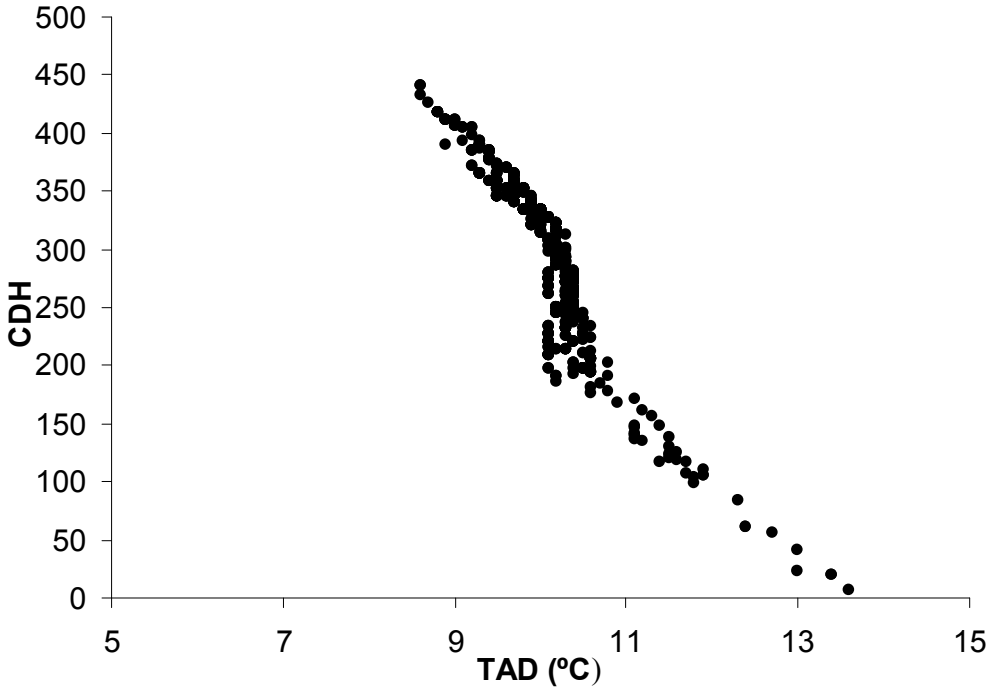

Figure 4. Relationship between cooling degree hours (CDH) and temperature at death (TAD) for *Oreochromis niloticus* juveniles.

4. Discussion

The TAD values observed in this study correspond well with those reported in earlier studies on *O. niloticus*. Mortality for the Egyptian strain has been reported from 13 ºC to 10 ºC (Lahav and Ra'anan, 1998) and from 11 ºC to 9 ºC (Khater and Smitherman, 1988). However, Sifa et al., (2002) reported better cold tolerance with first mortality at 11 ºC and total mortality at 7.4 ºC for the Egyptian strain of *O. niloticus* used in China. Several studies have shown that the degree of tolerance to lethal temperatures is dependent upon environmental effects, history of the fish and genetic effects (Cnaani et al., 2000) as well as fish health and nutrition status. The lower TAD values reported by Sifa et al. (2002) may have been due to the larger size of the tested fish (41-144 g compared to 1-20 g in this study). Furthermore, their fish had been reared under lower temperature for several generations. As reported for many ectotherms (Cossins and Bowler, 1987), both acclimatization and acclimation can extend the thermal tolerance

range of organisms.

We observed significant differences among families (Table 2) suggesting the existence of genetic variation for cold tolerance. The base population was bred from fish originating from four different parts of Egypt. These populations also exhibited differences in growth performance (Elghobashy, 2001; Rezk et al., 2002). Studies have shown that intra-specific evolution of thermal sensitivity is related to growth rate in fish. Generally, fish from higher latitudes grow relatively faster at lower temperatures than fish from low latitudes (Angilletta et al., 2002). Determination of the nature and presence of trade-offs between the two traits may be a first step towards simultaneously selecting for both growth and cold tolerance.

We found a significant aquaria effect on cold tolerance in this study (for example with CDH as response variable, the BLUE (estimated from Model 2) ranged from -11.6 to +24.5). However, aquaria did not differ in either their water quality or temperature. The significant aquarium effect may have been caused by aquaria position relative to the chiller in the cold room. The two aquaria that were exposed to direct cold draught also recorded relatively lower mean CDH values. This indicates the existence of a substantial effect of direct cold currents. Cold winds have been reported, in Taiwan, as a major cause of mass mortalities of cultured tropical fish including milkfish and tilapia (Wu et al., 1998). This suggests that the practice of controlling winter winds by some fish farmers may have a positive effect on fish over-winter survival.

The existence of size-dependent over-winter mortality has been reported for many freshwater and marine fishes, with smaller individuals being more susceptible than larger conspecifics (Sogard, 1997). The effect of size on cold tolerance has been reported as non-significant for several tilapia species and their hybrids (Behrends et al., 1990; Cnaani et al., 2000; 2003). However, Hofer and Watts (2002) noted that smaller fingerlings, below 5 g, were more susceptible to acute exposure to lower temperatures stress. Atwood et al. (2003), working with larger fish indicated that size significantly affected cold tolerance in *O. niloticus*. The very high marginal mean square effect for body weight in this study (Table 2) indicates a strong impact of weight on cold tolerance. Our study also indicates that smaller fish (<5g) are more susceptible (Figure 3).

Cold tolerance as a trait has been variously described. It has been described as low lethal temperature or temperature at death (TAD) (Khater and Smitherman, 1988; Behrends et al., 1990; Atwood et al., 2003). It has also been described as cooling degree days (CDD) or cumulative degree hours (CDH) (Behrends et al., 1990; Cnaani et al., 2000; Atwood et al., 2003) or simply as "number of days until death" (Tave et al., 1989). CDD and CDH incorporate time and temperature at which death occurs in such a way as to distinguish fish that die at the same temperature but at different times. This makes them more precise than TAD and number of days until death. CDH gives a better determination of cold tolerance than CDD because temperature is measured at shorter time intervals. The high correlation between the hourly TAD and CDH values indicates that both describe cold tolerance in a similar manner. CDH however, has a better resolution (and higher variation) than TAD and should be used for description of quantitative genetic parameters for cold tolerance.

Results of this study indicate low (0.08) heritability for cold tolerance trait in *O. niloticus* juveniles. These estimates are lower than the realized heritability estimates of 0.33 for *O. aureus* and 0.31 for the *O. aureus* X *O. niloticus* hybrids obtained by Behrends et al. (1996). However, they are within the range of the realised estimates of -0.05 to 0.31 for *O. niloticus* (Behrends et al., 1996). Estimates of realized heritability can be obtained from selection experiments using the response/ selection differential ratio or the standard least square procedures (Becker, 1984). Such estimates may be biased by failure to account for genetic or environmental sources of variation like maternal effects (Hill, 1972a, b; Johnson et al., 2002). In this study we found a substantial amount of common environmental/ full-sib effects. A strong maternal component of cold tolerance has been reported for *O. aureus* and its hybrids (Lee, 1979). Tave et al. (1989) reported a slight effect of egg cytoplasm in increasing cold tolerance but reported insignificant strain maternal effects in *O. niloticus*. When the CDH and TAD were corrected for body weight in this study, the common environmental/ full-sib effect was significantly reduced. This effect is likely to have increased due to body weight differences.

This study indicates low heritability and substantial common environmental or maternal effects on cold tolerance in *O. niloticus*. In the present study common environmental and maternal effects are confounded and therefore we cannot distinguish between them. Considering earlier

work (Tave et al., 1989), maternal effects may have been present in this study. This indicates that to precisely predict cold tolerance of tilapia progeny a model containing direct additive and common environmental/ full-sib or maternal effects rather than only direct effects is most appropriate. Since c^2 is expected to reduce with time, this aspect might affect heritability estimates for larger fish. However, mortality of juveniles is of a greater concern in tilapia culture and therefore interest is in heritability estimates in juveniles. It has been shown that rearing conditions can have a significant impact on cold tolerance of tilapia (Cnaani et al., 2003; Charo-Karisa et al., 2004). Since hapa effects increase c^2 for body weight and consequently cold tolerance, rearing juveniles in a single large hapa or pond could reduce c^2. Cold tolerance can be enhanced by improvement of husbandry practices (environmental or dietary) that ensure optimal fry body weight before onset of winter. We could not obtain accurate estimates for genetic correlation between cold tolerance and body weight from our data probably due to the limited number of observations. Cnaani et al. (2003) found significant association between two QTL for cold tolerance and body weight within the same linkage group but the two QTL were approximately 22cM apart. Results from the present study suggest that selection for higher body weight does not have any negative consequences for cold tolerance. However, genetic correlations could not be estimated very accurately and further research is needed to more accurately ascertain the genetic relationship between these traits.

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Chapter 4

Effects of rearing conditions on low-temperature tolerance of Nile tilapia (*Oreochromis niloticus)* juveniles

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Abstract:

This paper summarizes the results of two experiments in which the effects of genotype, age, size, condition factor and diet (natural phytoplankton versus formulated protein pellets) on low-temperature tolerance of juvenile *Oreochromis niloticus* were studied. The experiments were conducted at the WFC experimental facilities in Abbassa, Egypt.

In the first experiment, 775 juveniles from 43 sires and 80 dams were reared under mid-summer conditions for 41-91 days. In the second experiment, 393 juveniles were produced by single-pair mating of 20 dams and 20 sires from the same brooders as in the first experiment. These fish were reared for 42 days under autumn conditions with either high protein (40%) pellets or natural tilapia diet. At the end of the growth period fish from each experiment were tagged and exposed to gradually lowered temperatures. Cold tolerance was expressed as "temperature at death" (TAD) and cumulative degree hours (CDH). Cold tolerance was significantly affected by genotype, size, aquarium, condition factor (P= 0.0001) and diet (P=0.0547). In both experiments, smaller fish were more vulnerable to cold stress. Age did not significantly affect cold tolerance. Fish reared under mid-summer conditions died between 13.6 ºC and 8.6 ºC while those reared under autumn conditions died between 11.7 ºC and 7.5 ºC. This suggests that acclimatization to lower temperatures before cold stress can improve the cold tolerance ability of *O. niloticus.*

Key words: cold tolerance, acclimatization, diet, juveniles, *Oreochromis niloticus*

1. Introduction

One major constraint to the global expansion of tilapia farming is their sensitivity to low ambient temperatures. Of the tilapiine species, Nile tilapia (*Oreochromis niloticus L.*) is the most important, constituting 90% of all tilapia cultured outside Africa. Exposure to extreme cold temperatures leads to mass mortality (Chervinski and Lahav, 1976) making over-wintering a serious economic challenge. In fish, the degree of tolerance to lethal temperatures is dependent upon environmental effects, history of the fish and genetic effects (Cnaani et al, 2000) as well as fish health and nutrition status. It has been reported for many ectotherms that animals can extend their thermal tolerance range through acclimatization and acclimation (Cossins and Bowler, 1987). In tilapia, prior acclimation temperature and rate of temperature reduction are considered important factors determining mortality at a given temperature

(Stauffer, 1986; Stauffer et al., 1988). It is thought that the ability of fish to adapt to different temperatures is closely linked to the lipid composition in their muscles (Hazel, 1984; Greene and Selivonchick, 1987). Fatty acid composition is in turn influenced by the fish's diet (Henderson and Tocher, 1987). Kelly and Kohler (1999), working with bass reported that fish fed their natural diet suffered no mortality when exposed to simulated cold fronts while those fed on a prepared diet had 50-90% mortality.

In this paper, we present the results of two experiments in which the effects of genotype, age, size, condition factor, and diet (natural phytoplankton versus formulated protein pellets) on low-temperature tolerance of juvenile *Oreochromis niloticus* were studied. The experiments were conducted in two different periods of the year at the World Fish Centre, Abbassa, Egypt.

2. Materials and Methods

2.1 Experiment 1

This experiment was carried out during warmer temperatures at the beginning of summer (June-July 2003). Fish were produced in a full-sib/half-sib mating design in which each sire was mated to two dams and each dam mated to only one sire. A total of 80 full-sib families were produced from 43 sires and 80 dams. Sixty fry from each full sib family were reared in 80 separate 2 x 3 m hapas until they were tagged. The hapas were fixed in two 1000 m^2 ponds. Fry were 41-91 days old at the beginning of the cold tolerance challenge. Ten healthy individuals from each full-sib family were tagged with Floy tags between the dorsal fin and lateral line and used in the cold tolerance challenge. Individual body weights and standard lengths were recorded.

2.2 Experiment 2

This experiment was carried out in fall (September-October) of 2003. Twenty full-sib families were produced by single-pair mating of 20 dams and 20 sires chosen randomly from the brooders used in the first experiment. Experimental fish were reared in separate 6 m^2 hapas up to swim-up. Two groups of 30 swim-up fry each were obtained from each family and randomly assigned to two treatments described below. The growth experiment was carried out in a 4000 m^2 pond. The pond was fertilized with chicken manure at the rate of 50

kg $ha^{-1}day^{-1}$. Two rows of 20 (2 m X 1 m) hapas were placed in opposite ends of the pond. Fish in one row could feed only on naturally available food (Bowen, 1982) and phytoplankton induced by the chicken manure application. In the other row, fish were in addition fed twice daily (9.00 and 13.00 hrs) with 40% formulated protein pellets at 30% of their body weight. Fish were sampled on day 14, 21, 28, 35 and 42. In each sampling day, fry were counted, bulk weighed and average (family) weight recorded. On day 42, individual body weights and standard length measurements were also taken. Next, 20 randomly chosen fry from each full-sib family (10 per treatment) were tagged and used for the cold tolerance challenge.

2.3 Fish condition and growth

Fulton's condition factor was computed for each individual by the formula: $CF = 100W/L^3$ (Ricker, 1975), where W= body weight and L= body length. Specific growth rate (SGR; Experiment 2 only) was calculated according to Cho & Kaushik (1985): (ln final weight – ln initial weight)/time (days).

2.4 Cold tolerance challenge

After one day in ceramic tanks at ambient temperature, individuals from each family were randomly assigned to any of five 450 L glass aquaria set in a cold room. The room was served by a thermostatically controlled chilling unit. Each aquarium was constantly aerated using three air-stones connected to an air-pump. The temperature of the aquarium water was adjusted to the desired level by adjusting the compressor settings of the chiller. Fish were acclimatized to these aquarium conditions for 48 hours at 20 ºC. During the cold tolerance challenge, fish were not fed.

Following acclimatization, the temperature was first lowered to 16 ºC within 48 hours, and then to 11 ºC within the next 48 hours. From then on, water temperature was reduced at the rate of 1°C per day. Death was defined as the point at which fish lost balance, fell on their side and ceased fin, body and opercula movements and lost response to external stimuli. Dead fish were removed from the tanks at the end of each hour with a scoop net, and their tag and aquarium numbers recorded. Cold tolerance was quantified as cooling degree hours (CDH) (Behrends et al., 1996) and temperature at death (TAD). CDH represents the sum of hours the

fish survived multiplied by the difference between the hourly and initial temperature for each fish. As in earlier studies (Behrends et al., 1996; Cnaani et al., 2000, 2003), the initial temperature for calculation of CDH was 16 ºC.

Aquarium water temperature was monitored hourly from beginning to end of the experiment. DO, temperature and pH were measured once a day with WTW® multi 340i meter. To maintain water quality within acceptable levels, total ammonia, nitrate and nitrite, were measured daily with HACH kits. Aquaria were cleaned twice daily by suction to remove faeces. Water that was removed during aquarium cleaning was replaced with clean water that had been pre-cooled with ice cubes.

2.5 Data analysis

All analyses were carried out using SAS software (SAS, Institute, Cary, NC, USA). Factors affecting cold tolerance in the first experiment were analysed by analysis of variance with the generalised linear model including sire, dam, aquarium, age, and size effect using the following model.

$$Y_{ijkl} = \mu + a_i + \beta_1 * d_{ijk\,l} + \beta_2 * ln\,(w)_{\,ijkl} + s_j + d_k\,(s_j) + e_{ijkl} \qquad \text{(Model 1)}$$

Where Y_{ijkl} = cooling degree hours for individual l; μ = overall mean; a_i = fixed effect of aquarium (i = 1, 2, 3, 4, 5); β_1 = regression coefficient of cooling degree hour on age; d_{ijkl} = a co-variable of age of the individual l; β_2 = regression coefficient of colling degree hours on natural logarithm of body weight; $ln(w)_{ijkl}$ = a co-variable of the natural logarithm of body weight of individual l; s_j = effect of the jth sire; $d_k(s_j)$ = effect of the kth dam nested within the jth sire; and e_{ijkl} = random residual effect associated with individual l.

In the second experiment, the effects of diet, genotype, aquarium, body weight, standard length, specific growth rate, condition factor, and the first order interaction between genotype and diet were analyzed. Because specific growth rate did not affect cold tolerance, it was removed from the model. As in Model 1, the natural logarithm of body weight was used instead of body weight. The following model was finally fitted

$$Y_{ijkl} = \mu + a_i + g_j + t_k + \beta_1 * ln\,(w)_{ijkl} + \beta_2 * c_{ijkl} + g_j * t_k + e_{ijkl} \qquad \text{(Model 2)}$$

Where Y_{ijkl} = cooling degree hours for the lth individual; μ = overall mean; a_i = fixed effect of aquarium (i = 1, 2, 3, 4, 5); g_j is the effect of the jth family; t_k is the effect of diet $(k = 1, 2)$; β_1 and β_2 are the regression coefficients of cooling degree hours on body weight and condition factor respectively; lnw_{ijkl} is a co-variable of natural log of body weight of the lth individual; c_{ijkl} is a co-variable of condition factor of the lth individual; and e_{ijkl} is a random residual effect associated with the individual l.

3. Results

3.1 Experiment 1

Means and standard deviation of body weight (BW) and length (SL), condition factor (CF), temperature at death (TAD) and cooling degree hours (CDH) are shown in Table 1. Size of fish ranged from 1 to 20.6 g body weight and 29.6 to 78 mm standard length. Fish died due to cold from 13.6 ºC to 8.6 ºC and had a mean cooling degree hours of 298. The GLM analysis from Model 1 showed significant effects ($P < 0.0001$) of body weight, and genotype (sire and dam) on cold tolerance. Age did not significantly affect cold tolerance. There was a tendency for smaller fish to have lower CDH values suggesting that size affects the ability of fingerlings to survive low temperatures. The correlation coefficient of CDH on body weight was low but significant (0.58, $P = 0.0001$). The relation between body weight and CDH was logarithmic with an inflection point around 5 g.

Table 1. Overall means and standard deviations of body weight, standard length, age and cold tolerance responses of *Oreochromis niloticus* juveniles exposed to experimentally lowered temperatures

Trait	Mean	Std. deviation	Minimum	Maximum
Body weight (g)	5.10	2.35	1.0	20.6
Standard length (mm)	50.61	7.60	29.6	78.0
Age (days post-hatch)	79.00	8.62	41.0	91.0
Temperature at death (ºC)	10.10	0.56	8.6	13.6
Cooling degree hours	298.07	67.86	6.4	440.3
*LT_{50} (full-sib) (ºC)	10.10	0.37	9.3	11.5
LT_{50} (half-sib) (ºC)	10.10	0.24	9.4	11.1

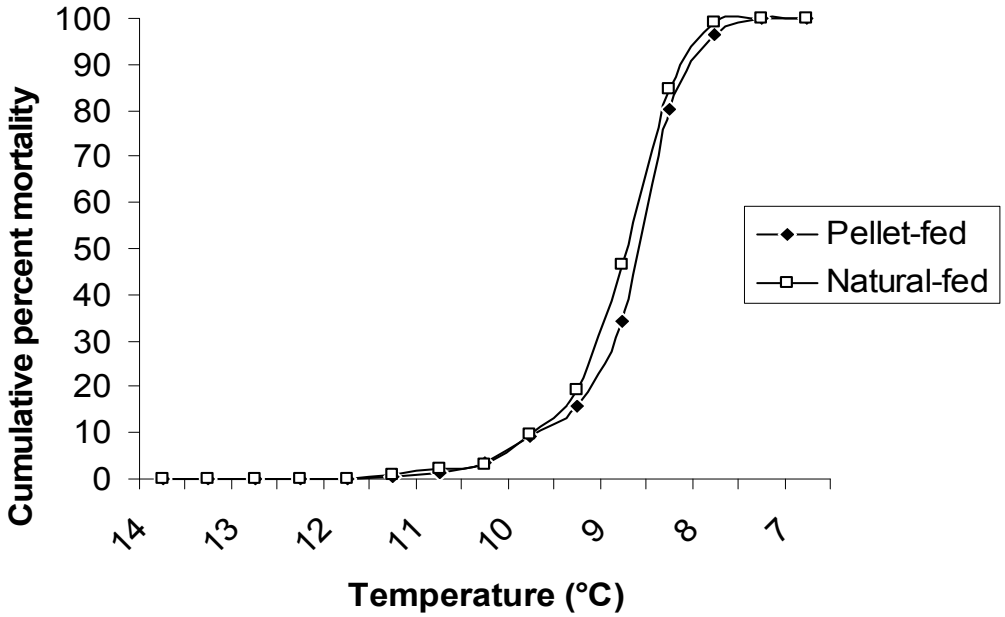

Figure 1. Mortality rate of *Oreochromis niloticus* juveniles exposed to reduced temperatures. Fish had been grown under either pellet-fed (40% protein formulated pellets) or natural-fed (chicken manure only) conditions.

3.2 Experiment 2

Mortality of fish from the two treatments with lowering of temperature is shown in Figure 1. The natural-fed fish started dying at 11.7 °C while the pellet-fed group begun dying at 11.5 °C.

Means and standard deviation of body weight (BW) and length (SL), condition factor (CF), specific growth rate (SGR), TAD and CDH within diet treatments are shown in Table 2. The lowest TAD at which all fish died was 7.5 °C and 7.6 °C for the pellet-fed and natural-fed fish respectively. Fish from the two treatments differed significantly with respect to CF (P = 0.001) and cold tolerance (when expressed as TAD (P= 0.035) or CDH (P= 0.041)). There were no significant differences in SGR (P= 0.8659), BW (P= 0.4771) or SL (P= 0.2239) between the two diet groups although these values were slightly higher for the pellet-fed fish. Cold tolerance was significantly affected by size (BW), condition factor, family, aquarium (P= 0.001) and diet (P= 0.0547). We found a significant interaction between family and diet for cold tolerance (P= 0.011). Pellet-fed fish had generally higher CDH values, but in some families natural-fed fish had higher CDH values (Figure 2).

Table 2. Means and standard deviations of body weight, standard lengths, specific growth rate, condition factor, and subsequent temperature at death and cooling degree hours of juvenile *O. niloticus* reared for 42 days on different diets

Parameter	Diet		
	Pellet-fed	Natural-fed	P-value
Initial weight (g)	0.05 (0.03)	0.05 (0.03)	-
Final weight (g)	1.97 (0.65)	1.92 (0.61)	0.477
Standard length (mm)	38.05 (3.99)	37.58 (3.81)	0.224
Specific growth rate (%/day)	9.37 (1.21)	9.34 (1.29)	0.866
Condition factor	3.86 (0.40)	3.71 (0.37)	0.001
Temperature at death (ºC)	8.90 (0.67)	9.00 (0.64)	0.035
Cooling degree hours	551.66 (104.53)	530.56 (99.80	0.041

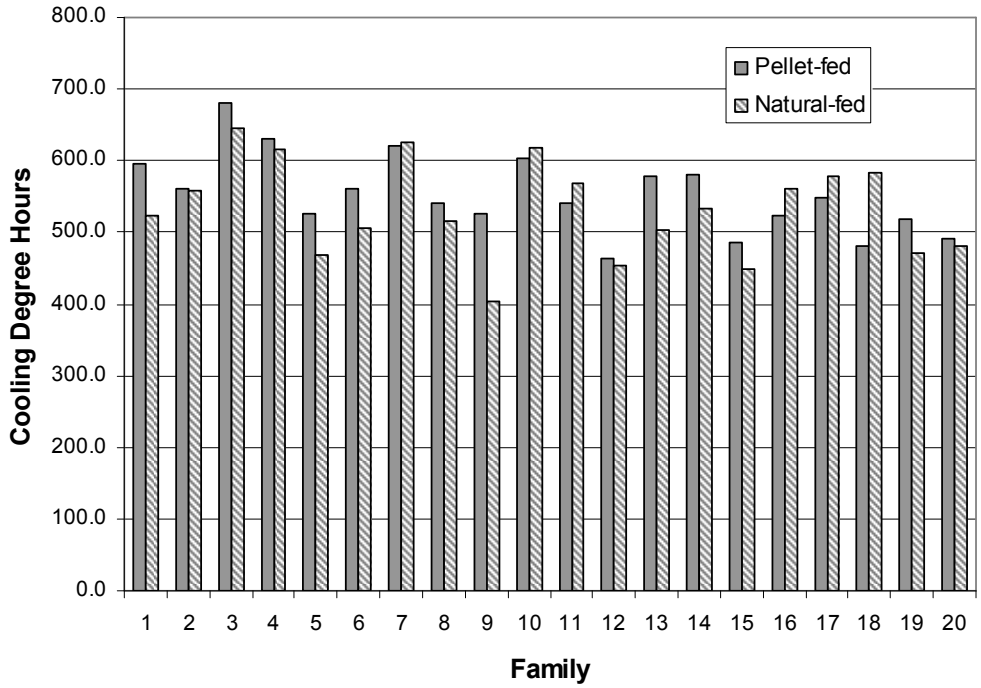

Figure 2. Least square means of CDH among families of *Oreochromis niloticus* reared in two treatments: chicken manure and 40% protein pellets.

3.3 Comparison of Experiment 1 and 2

The temperature profiles during Experiment 1 and 2 are shown in Figure 3. Temperature ranged from 21.4–28.4 ºC (minimum readings) and 24.3–33.1 ºC (maximum readings) in the summer period and from 20.3–24.1 ºC (minimum) and 23.6–29.1 ºC (maximum) in the autumn period.

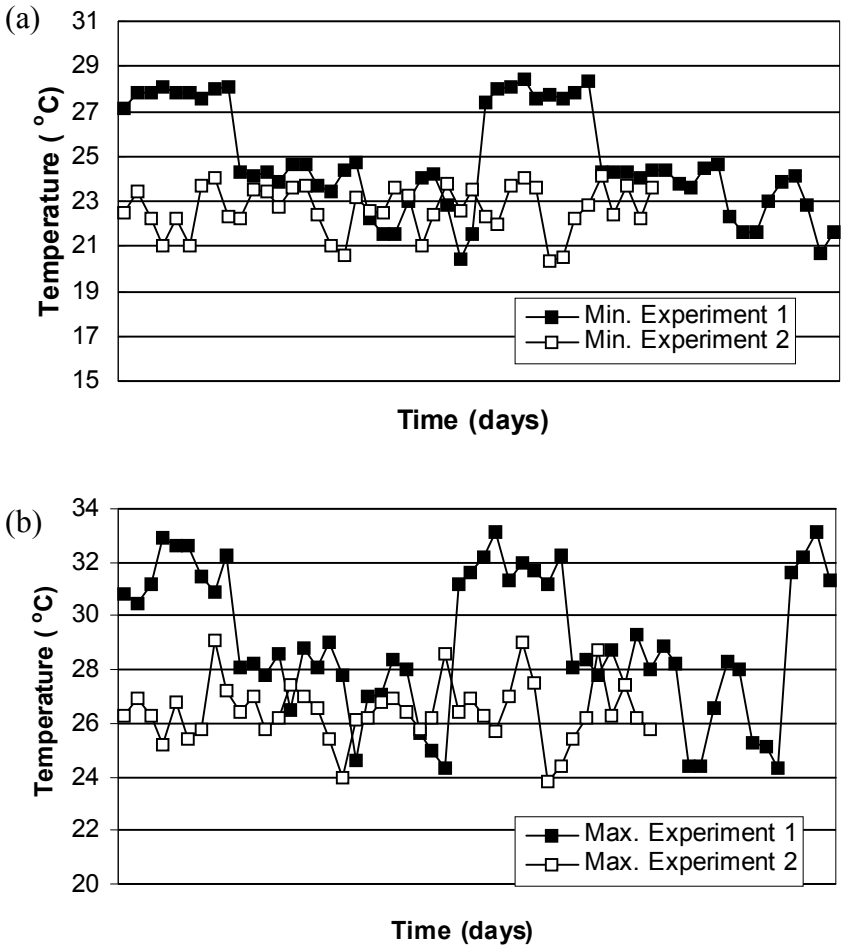

Figure 3. Temperature profiles during the rearing period of two groups (Experiment 1 and 2) of *Oreochromis niloticus* juveniles before exposure to lowered temperatures. Chart (a) and (b) show morning and afternoon temperature readings respectively. Experiment 1was carried out under summer conditions and Experiment 2 in autumn.

The combined results of TAD, fish size and CDH for the two experiments are shown in Table 3. To produce the values for Experiment 2 in Table 3, measurements of fish from the two dietary treatments in Experiment 2 were pooled. Fish began to die from 13.6 ºC to 8.6 ºC in Experiment 1, and from 11.7 °C to 7.5 °C in Experiment 2 (Figure 4). In Experiment 1, mean TAD and CDH were 10.1 ºC and 298.1 respectively while in Experiment 2, TAD and CDH were 9 °C and 541.1 respectively indicating that fish in Experiment 2 were more cold tolerant.

Table 3. Means and standard deviations of body weight, standard lengths, temperature at death and cooling degree hours of juvenile *O. niloticus* in Experiment 1 and 2

Parameter	Experiment 1		Experiment 2	
	Mean (SD)	Range	Mean (SD)	Range
Body weight (g)	5.1 (2.33)	1.0 - 20.6	2.0 (0.63)	0.8 - 4.7
Standard length	50.8 (7.35)	29.9 - 78.0	37.8 (3.90)	27.4 - 51.2
Temperature at death	10.7 (0.56)	8.6 - 13.6	9.0 (0.66)	7.5 - 11.7
Cooling degree hours	298.1 (67.86)	6.4 - 440.3	541.1 (102.6)	181.8 - 763.0

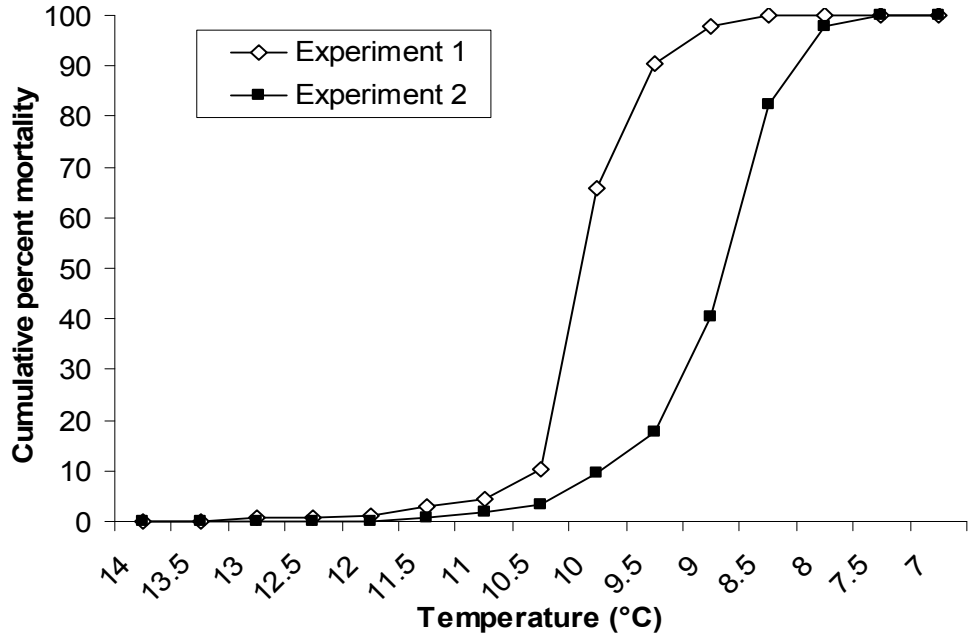

Figure 4. Mortality rate of *Oreochromis niloticus* juveniles under reduced temperatures. Fish reared under high (Experiment 1) and low (Experiment 2) ambient temperature regimes.

4. Discussion

Temperature at death values of between 13.6 ºC to 8.6 ºC in experiment 1 and 11.7 °C to 7.5 °C in experiment 2 are comparable with earlier findings on cold tolerance of *O. niloticus*. The Egyptian strain has been reported to experience mortality from13 ºC to 10 ºC (Lahav and Raanan, 1998) and from 11 ºC to 9 ºC (Khater and Smitherman, 1988). Slightly better cold tolerance of between 11 ºC and 7.4 ºC have been reported for the Egyptian strain of *O. niloticus* used in China Sifa et al. (2002).

The existence of size-dependent over-winter mortality has been reported for many freshwater and marine fishes, with smaller individuals being in most cases more susceptible than larger con-specifics (Sogard, 1997). The effect of size on cold tolerance in tilapia has been reported as either significant or insignificant by different authors (Behrends et al 1990; Cnaani et al 2000; Cnaani et al, 2003). Atwood et al. (2003), working with larger fish indicated that size significantly affected cold tolerance in *O. niloticus.* Our study indicated that size affected cold tolerance with smaller fish (<5g) being more susceptible. This confirms the observation by farmers that smaller fish are more vulnerable during winter months. For better over-winter survival, juvenile of Nile tilapia should be at least 5g in size.

Diet has been known to improve the ability of fish to tolerate low temperatures. For example for bass, Kelly and Kohler (1999) reported that fish fed their natural diet suffered no mortality and had higher levels of unsaturated lipids than those artificially fed. Similarly, dietary supplementation of L-carnitine at different levels led to higher cold tolerance in an ornamental cichlid, *Pelvicachromis pulcher* (Harpaz et al., 1999). It has been shown that the level of dietary protein affects lipid content of muscles and liver in Nile tilapia (Ogunji and Wirth, 2002) and its hybrids (*O. niloticus* x *O. aureus*; Chou et al., 2001; Huang et al, 1998). Atwood et al. (2003) found that when *O. niloticus* were fed either menhaden oil or coconut oil diets, they incorporated differing levels of saturated (n-6) or unsaturated (n-3) fatty acids into their muscles. However, this did not significantly affect cold tolerance of the two fish groups (Atwood et al., 2003). In the present study, fish fed protein pellets had significantly higher mean cold tolerance than natural-fed fish.

Our study indicated a significant effect of condition factor on cold tolerance. Since the pellet-fed group had higher condition factor, their higher cold tolerance may be attributed to their having better condition. Morphometric indices which assume that heavier fish of a given length are in better condition are simple indicators of energy storage (Lloret et al, 2002). Fish condition or well-being has a large influence on growth, reproduction and survival of fsih populations (Lambert and Dutil, 2000). It appears that by improving fish condition factor, one can increase winter survival of *O. niloticus* to some degree. It should be noted however that judging from the the TAD values, this difference may not practically improve survival at the farm during severe winters.

We observed that significant family by diet interaction effects. Some families had higher cold tolerance ability after growth with either natural-fed or formulated pellet diets. This within family variation with respect to diet may indicate the presence of a genotype X environment interaction in cold tolerance of Nile tilapia.Significant genotype by diet interaction may point to the need for particular genotype-diet combinations for better low temperature tolerance. The significance of genotype by diet interaction for cold tolerance in tilapia should be further studied.

In this study, fish grown under lower autumn temperature showed better tolerance to exposure to lower temperatures as shown in Figure 4. This confirms the hypothesis that prior acclimatization to lower temperature conditions can lead to better tolerance to low temperatures in tilapia (Stauffer, 1986; Stauffer et al., 1988). Temperature in conjunction with photoperiod has been shown to affect fish physiology. Survival, growth rates and feed utilization of *O. niloticus* fry are affected by changes in photoperiod (El-Sayed and Kawanna, 2004). Atwood et al. (2003) showed that for Nile tilapia, the effect of experimentally altering photoperiod alone has some effect on cold tolerance when the trait was expressed as cooling degree hours (CDH) but not when expressed as temperature at death (TAD).

In this study, acclimatization to lower temperature not only improved CDH values but also led to substantial decrease in TAD values indicating better cold tolerance. While it is not possible, based on this study, to point out the exact factors that led to the improved cold tolerance in the second experiment, prior low temperature acclimatization coupled with photoperiod may have played a key role. The large differences in cold tolerance in the two experiments point to a possibility of using prior low temperature acclimatization conditions before fish over-wintering as a tool for improvement of cold tolerance in Nile tilapia.

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Chapter 5

Heritability estimates and response to selection for growth of Nile tilapia *(Oreochromis niloticus)* in low-input earthen ponds

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Abstract

This study presents results of two generations of selection (G_1 and G_2) for growth of Nile tilapia. The selection environment consisted of earthen ponds which were daily fertilized with 50 kg dry matter (dm)/ ha chicken manure. No supplementary feeds were provided. In total, 6429 fully pedigreed experimental fish were included in the analysis. Survival till harvest was highly variable ranging from 35% to 77% and was affected by initial weight, pond, and age effects. Body weight at harvest (BW) increased from a mean of 67.4 g in the grandparental (unselected) population (G_0) to 129.5 g in G_2 was affected by initial weight, pond, sex and age effects. Generations were discrete and therefore genetic parameters were estimated separately for each year. Heritability estimates for BW ranged from 0.38 to 0.60, and the heritability for survival ranged from 0.03 to 0.14. The estimated selection response was 23.4 g (34.7%) between G_0 and G_1 and 13.0 g (14.9%) between G_1 and G_2. These results demonstrate the feasibility of selection for growth of Nile tilapia in low-input environments.

Keywords: Nile tilapia; *Oreochromis niloticus*; Selection response; Body weight; Heritability; Breeding value

1.0 Introduction

Tilapias are, after carp, the second most important group of farm raised fish in the world. They are the mainstay of many resource-poor fish farmers (Eknath et al., 1993). Among the tilapiines, the Nile tilapia (*Oreochromis niloticus* L.), is the most important cultured fish species. Although *O. niloticus* is farmed in a wide range of aquaculture systems (Pullin, 1985), majority of its culturing is carried out in the tropics in semi-intensive environments such as fertilized earthen ponds. Nile tilapia is herbivorous by nature, consuming mainly phytoplankton (Moriarty, 1973; Moriarty and Moriarty, 1973), but can as well consume a variety of other natural food organisms found in ponds (Bowen, 1982). To increase fish production, supplementary or artificial feeds may be added. However, supplementary feeds can take up to 60% of fish production costs (Green, 1992) making them unaffordable for most farmers in the developing countries (Nguenga et al., 1997; Liti et al., 2005). Due to the high cost of supplementary feeds, poor farmers either grow Nile tilapia with organic fertilization alone or with a variety of locally available farm resources. This leads to reduced yields and small fish sizes at harvest.

A number of selective breeding programs have been initiated to improve the growth of *O. niloticus* in ponds and cages (e.g. Hulata et al., 1986; Eknath et al., 1993; Bensten et al., 1998). Initial trials at selective breeding, which were based on mass selection for growth, indicated low response to selection for growth (Tave and Smitherman, 1980; Hulata et al., 1986; Teichert-Coddington and Smitherman, 1988; Huang and Liao, 1990). Recently, considerable improvement of response to growth has been achieved using family selective breeding schemes and tilapia germplasm assembled from several wild stocks in Africa (Eknath et al., 1998). These selection programs have typically been carried out in relatively favorable environments receiving supplementary feed. However, there are reports that the gains of selection in Nile tilapia were lost when selected breeds were tested in less favorable environments (Macaranas et al., 1997). This could indicate that the expression of body weight in different environments, i.e. low and high-input culture conditions, is influenced by a different set of genes.

Here we report on the analysis of a Nile tilapia selection experiment carried out in ponds receiving chicken manure as the only external nutrient source. The aim was to estimate heritability for growth and survival and to investigate the potential of selecting for growth in low-input environments (i.e. manure fertilized ponds without supplementary feeding).

2.0 Materials and Methods

The experiment was carried out at the Regional Center for Africa and West Asia of the World Fish Center, Abbassa, Egypt. Fish used for this study were the G_0 population produced in 2002, the first generation of selection (G_1) produced in 2003, and the second generation of selection (G_2) produced in 2004.

2.1 The founder population and production of G_0

The founder population (i.e. parents of the G_0 population) was produced in spring of 2000 in a full diallel mating design among local Egyptian strains namely Maryout, Zawia, Abbasa and Aswan (Rezk et al., 2002; 2004). Eighty sires and 105 dams, selected at random from among the founder stock, were subsequently used to produce the G_0. Each sire was mated to two dams and each dam mated to only one sire, thus generating full and half sib groups. Fry were raised in 2 x 1 x 1 m hapas suspended in concrete tanks and were fed twice daily with

40% protein supplements, initially in the form of powder and later as pellets. Initial feeding rate was 20% of body weight, which was gradually reduced to 5% body weight at tagging size (i.e. mean wet weight of 2g).

2.2 Production of G_1 and G_2

The first and second generations of selection, G_1 and G_2, were produced in 2 x 3 x 1 m hapas suspended in fertilized ponds. Each sire was mated to two dams as in G_0. Fifty sires and 87 dams were used to produce generation G_1, while for generation G_2, 54 sires and 104 dams were used. At first, each sire was kept in a single hapa with two dams. Twice a week, hapas were checked for occurrence of spawning. Spawning was assumed to have occurred when the dam had eggs or yolk-sac fry in her mouth. The un-spawned dam and the sire were both transferred to an adjacent hapa thus producing the paternal half-sibs. To prevent multiple spawning, the male was removed immediately after spawning occurred. When swim-up fry were sighted in both hapas, the females were also removed. Two to three weeks later, the number of swim-up fry in each hapa was reduced to 80 individuals. In contrast to the G_0, the G_1 and G_2 fry were given no supplementary feeds and were reared in hapas suspended in the earthen ponds. To boost natural pond productivity, ponds with hapas containing fry were fertilized daily with chicken manure at the rate of 50 kg dry matter, dm /ha.

2.3 Grow-out and pond management (G_0, G_1 and G_2)

As soon as a family reached suitable tagging size, 24 randomly chosen fry from each full-sib family were individually tagged with Floy® tags and returned into the hapas until stocking. Fry were between 31-96 days old at stocking. Each family of fry was randomly divided into two groups which were then stocked in two 1000 m^2 fertilized earthen ponds for grow-out. Ponds were daily supplied with dry chicken manure from layer and broiler farms at the rate of 50-kg dm/ha. This fertilization rate corresponds to 0.3 kg nitrogen ha^{-1} day^{-1} which is enough to support yields of 4.3 kg fish BW ha^{-1} day^{-1} (calculated from Knud-Hansen et al., 1991). Fish that died within the first week of stocking were, when possible, replaced with individuals from the same family. Pond water levels were maintained by weekly addition of water. Water quality parameters (temperature, pH, and dissolved oxygen (DO)) were monitored daily in the first and second years, and twice a week in the third year. After approximately 8 months of growth, which included 3-4 months of over-wintering, fish were

harvested by seine netting. Fish were on average 289 days old at harvest in G_0, 293 days old in G_1, and 318 days old in G_2.

2.4 Selection procedure

Before measurements were taken, fish were anaesthetized with MS222 (3-aminobenzoic acid ethyl ester) to avoid handling stress. Each fish was measured for body weight (BW) and their breeding values estimated from an animal model (Model 1 below). Brooders were selected based on the rank of EBVs, separately for males and females. These estimates were used to select 100 males and 200 females as potential brooders for the next generation. We aimed at spawning the best ranking 50 males and 100 females. However, to minimize inbreeding, the number of individuals from a single full sib or half sib group was restricted which made fish that were not among the best ranks to be included as brooders.

2.5 Data Analysis

Because, the generations were discrete i.e. sires and dams were used only one generation, data from each generation (G_0, G_1, and G_2) were analyzed separately. A complete pedigree of the experimental fish from G_0 onwards was available and was used in each analysis. An animal model including common environmental/ full-sib family effect was used:

$$Y_{ijkl} = \mu + \mathrm{PND}_i + \mathrm{SEX}_j + \beta_1 * AGE_{ijkl} + \beta_2 * log\ (INWT_{ijkl}) + \mathrm{d}_k + \mathrm{u}_l + e_{ijkl} \ldots. \text{ (Model 1)}$$

Where Y_{ijkl} is body weight of individual l at harvest; μ is overall mean; PND_i is fixed effect of pond (i = 1, 2); SEX_j is the fixed effect of sex (j = 1, 2); β_1 is the regression coefficient of body weight at harvest (BW) on age at harvest; AGE_{ijkl} is a covariate of age at harvest of individual *ijkl*; β_2 is a regression coefficient of BW on natural logarithm of initial body weight; $log(INWT_{ijkl})$ is a co-variable of the natural logarithm of initial body weight of individual *ijkl* (the natural logarithm was used instead of untransformed initial body weight values because body weight was logarithmically related to initial body weight); u_l is a random additive genetic effect of the lth individual; d_k is a random common environmental/ full-sib effect which included the effect of the dam, the effect of common hapa rearing, and a quarter of non-additive genetic effects; and e_{ijkl} is a random residual effect associated with individual *ijkl*. Breeding values for body weight of each individual were estimated from

Model 1. Because the main interest was in the growth that occurred after stocking in the pond, initial (= stocking) weight was included as a co-variable in the model.

Factors affecting survival at harvest were also analyzed separately for each year. Surviving fish were given a score of '1'; while dead fish and those that had lost tags, and hence could not be identified, were scored as '0'. The factors included in the model were: pond as a fixed effect, and initial body weight and age at stocking as co-variables. Sex effect on survival was not estimated because at the time of stocking it was not possible to score sex visually. Animal and dam were included in the model as random effects. Variance components for survival at harvest were estimated from a univariate model.

Total phenotypic variance (σ^2_P), was calculated as the sum of the additive genetic variance (σ^2_A), the common environmental/full-sib component of variance (σ^2_C), and the error variance (σ^2_E). The heritability (h^2) was computed as σ^2_A/σ^2_P, and the common environmental/full-sib effect (c^2) as σ^2_C/σ^2_P. All computations were carried out with ASREML (Gilmour et al., 2002).

2.5.1 Selection response

The selection differential for growth was estimated for each generation by comparing the mean estimated breeding value of the brooders that actually produced progeny and the mean of all the fish before the brooders were selected. The selection differential was calculated from the EBVs of males and females separately and then averaged over the sexes. EBV was calculated based on information available at the time of selection, i.e. phenotypic information from progeny was not used. The selection differential in G_0 and G_1 was then compared to the observed differences in mean BW between generations.

3.0 Results

3.1 Descriptive statistics

Mean BW in different ponds ranged from 63.2 and 71.5 g in G_0 to 128.1 and 130.9 g in G_2 (Table 1). The CVs were higher for initial body weight (46.7 - 87.9%) than for BW (30 - 56%). Survival to harvest ranged from 35 % to 77%, with Pond 2 recording consistently lower survival throughout the experiment. The realized final yields (from actual surviving

fish in each pond) were 344.5 to 1042.2 kg /ha per growth cycle. The expected final yields, which were calculated after correcting for mortality and stocking density, increased from 608.7 to 1264.5 kg /ha between G_0 and G_2.

In the study period, morning temperatures ranged from 16.3 to 27.5 °C, and afternoon temperatures from 17.5 to 31.9 °C, and morning pH ranged from 8 to 9.8 and afternoon pH from 7.9 to 10.8. Morning dissolved oxygen (DO) ranged from 0.6 to 6.5 mg/l, while afternoon DO from 6.5 to 20.1 mg/l. Water quality parameters were similar between ponds but differed between generations. For example, mean morning temperature was 22.5 °C in G_0, 24.6 °C in G_1, and 24.7 °C in G_2; afternoon temperature was 25.4 °C in G_0, 28.7 °C in G_1, and 27.8 °C in G_2. Morning DO was 2.1 mg/l in G_0, 3.4 mg/l in G_1, and 2.1 mg/l in G_2; and afternoon DO was 7.4 mg/l in G_0, 12.1 mg/l in G_1, and 10.8 mg/l in G_2.

Table 1. Number of fish stocked and harvested, survival and tag loss in each pond, mean initial weight and mean body weight at harvest (BW), with standard deviations (SD) and coefficients of variation (CV), and realized final yield (RFY) and expected final yields (EFY) of Nile tilapia in each generation

Generation	Pond*	Stocking				harvest						
		n	Initial weight (g)			%	%	BW (g)			Yield (kg/ha)	
			Mean	SD	CV	Survival	Tag loss	Mean	SD	CV	RFY	EFYϕ
G_0 (2002)	1	1058	2.2	1.9	84	72	11	71.5	38.9	54	528.6	692.7
	2	1064	2.3	1.8	79	53	3	63.2	35.6	56	344.5	608.7
G_1 (2003)	3	1058	4.3	1.8	41	70	7	70.0	23.5	34	485.8	657.3
	2	1043	3.7	1.5	41	59	3	104.4	32.6	31	618.2	1006.8
G_2 (2004)	3	1101	4.3	3.1	72	77	2	128.1	417	33	1042.2	1237.7
	2	1105	4.5	3.8	84	35	2	130.9	39.5	30	493.2	1264.5

*Pond numbers refer to a unique unit

ϕ Calculated from the difference between BW at harvest and initial body weight assuming a stocking density of 1 fish/ m^2 with no mortalities

3.2 Environmental effects

BW was affected by initial body weight, sex, age, and pond effects (Table 2). There were differences in magnitude of effects in different generations. For example, the magnitude of the effect of pond on BW relative to other effects was greatest in G_1 but lowest in G_2. The regression coefficients of BW on logarithm of initial body weight were positive for both

sexes in all generations. They ranged from 27.1- 41.0 for males and 20.2 to 36.4 for females. However, initial body weights were significantly larger for males than for females. Survival was affected by initial body weight, age, and ponds in different generations (Table 3).

Table 2. F-values from the type III sum of squares of effects on body weight at harvest in the G_0, G_1, and G_2 according to model 1

		G_0	G_1	G_2
Source	DF	F-value	F-value	F-value
Sex	1	303.0**	397.2**	657.4**
Pond	1	35.0*	1312.5**	0.1
INWT	1	376.0**	195.2**	622.5**
Age	1	10.8*	2.5	2.3

** P<0.01
*P<0.05

Table 3. F-values from the type III sum of squares of effects on survival at harvest

		G_0	G_1	G_2
Source	DF	F-value	F-value	F-value
Pond	1	34.92*	7.80	469.54**
Age	1	5.02*	32.20*	1.81
INWT	1	2.89	8.94*	0.03

** P<0.01
*P<0.05

3.3 Genetic parameters and selection response

The heritability (h2) estimates for body weight and survival are presented in Table 4. The h^2 estimates for BW was 0.60 in G_0, 0.38 in G_1, and 0.51 in G_2. The common environmental/full sib effect (c^2) was generally low, and ranged from 0.08 to 0.11. The phenotypic variance for BW, after adjustment of the observations for fixed effects in the model, was between 510 and 794 g^2. The heritability estimate for survival was 0.12 in G_0, 0.03 in G_1, and 0.14 in G_2.

Table 4. Estimates of total phenotypic variance (σ^2_P), heritability (h^2) and common environmental/ full-sib effects (c^2) with standard errors (SE) for body weight and survival at harvest

	Body weight			Survival		
Generation	σ^2_P	h^2	c^2	σ^2_P	h^2	c^2
G_0	782.2	0.60 (0.08)	0.09 (0.07)	0.2	0.12 (0.03)	0.00 (0.00)
G_1	510.0	0.38 (0.12)	0.11 (0.07)	0.2	0.03 (0.04)	0.04 (0.02)
G_2	794.0	0.51 (0.19)	0.08 (0.07)	0.2	0.14 (0.06)	0.01 (0.02)

The selection differential in growth was 23.4 g in G_0 and 13 g in G_1 (Table 5) corresponding to an average of 18.2 g per generation. The selection differential was calculated from EBVs and, therefore, it reflects the expected response to selection. The realized increase in BW from G_0 to G_1 was 19.8 g, which corresponds to 84.6% of the expected response to selection. The realized increase in BW from G1 to G2 was 42.3 g, which corresponds to 325% of the expected response.

Table 5. Selection differential (g) estimated by comparing EBVs of selected brooders and of all fish in each generation before selection, and the observed and expected means of body weight at harvest (BW)

Generation	Obs. BW	Differential	Exp. BW
G_0	67.4	23.4	-
G_1	87.2	13.0	90.8
G_2	129.5	-	100.2

4.0 Discussion

4.1 Coefficient of variation

In this study, the coefficients of variation were higher for initial body weight than for body weight at harvest (BW), indicating that younger fish have more variable body weights. A similar observation was made by Gjedrem (1983). Several authors suggest that the addition of supplementary feeds may lead to increased size variation by increasing competition for feed, especially if the feed is given at a central feeding point (Grant, 1993; Alanärä, 1996; Doupe and Lymbery, 2003; Rutten et al., 2005c). The large variation in initial body weight in the present study may have been due to competition for space and food because of hapa confinement in the nursing stage. The coefficients of variation for BW in this study were comparable to those in systems receiving pelleted feed (e.g. Rutten et al., 2005 a, b). Bentsen et al. (1998), found no differences in CV for BW of the same population of Nile tilapia in fertilized and fed ponds.

4.2 Environmental and sex effects

We found substantial effects of initial weight, pond and sex on body weight. The initial body weights for males were larger than for females in the present study indicating that sexual dimorphism in Nile tilapia starts at an early age. Several studies in Nile tilapia have shown that pond differences have a significant effect on body weight (Smart et al., 1997; Bentsen, et

al., 1998; Riley et al., 1998). In the present study, we observed differences in magnitude of pond effects in different generations. Eknath et al. (1993) observed similar changes in magnitude of environmental and sex effects in different generations, and attributed them to the wide range of environments used. Although we cannot find, based on our data, a single water quality parameter responsible for the differences in magnitude of pond effects between generations, these differences may be attributed to the sum of the differences in the water quality parameters between generations. The large differences in body weight and yield among ponds in this study emphasize the necessity of using replicate ponds in Nile tilapia selection experiments.

4.3 Survival to harvest

Widely varying survival rates (29-100%) have been recorded in Nile tilapia reared in fertilized ponds with or without supplementary feeds (e.g. Abdalla et al., 1996; Abdelghany and Ahmad, 2002; Bolivar and Newkirk, 2002), indicating that survival is an important trait to consider in pond experiments. Fertilized ponds are frequently affected by extended periods of low dissolved oxygen leading to hypoxia which may reduce growth (Chervinski, 1982) and cause mortality (Coche, 1982). In the present study, the lowest level of dissolved oxygen in the ponds was 0.6 mg/l. As suggested by Teichert-Coddington and Green (1993), it may be both the duration of hypoxic episodes and the level of dissolved oxygen rather than the level of dissolved oxygen alone that determine the effect of hypoxia on fish. During over-wintering in ponds, Nile tilapia frequently experience mortality in the study area (the Nile delta, Egypt). In a previous study (Charo-Karisa et al., 2005), we showed that small body weight (<5 grams) was a significant factor reducing cold tolerance of juvenile Nile tilapia. The variation in survival rates in this study was mainly due to pond and initial weight effects. Although our fish are likely to have been larger than 5g at the onset of winter, size effects may still have played a role in fish survival during the over-wintering period.

In the present study, we found a substantial amount of additive genetic variation and low common environmental effects for survival. The heritability (h^2) estimates of survival in this study are comparable with estimates of earlier studies in fish. For example, Standal and Gjerde (1987) and Rye et al. (1990) reported heritability estimates (0.04 to 0.21) for Atlantic salmon and Rainbow trout from the sire component and higher heritability from the dam

component of variance (0.25 and 0.87). Gjerde et al. (2004) found zero heritability and higher common environmental effects (0.12) for survival in Atlantic cod, suggesting non-additive genetic common environmental effects. In Nile tilapia reared in ponds, Eknath et al. (1998) reported lower heritability (0.08) compared to the present study. Murray et al. (1993) found differences in survival of the coastal and interior populations of Coho salmon. Fish in the present study had to go through an over-wintering period while those in Eknath et al. (1998) did not. Exposure to the cold over-winter period could explain the higher heritability found in our study. The h^2 estimates in the present study indicate prospects for improvement of this trait by selection. Although interesting, we could not obtain reliable estimates of correlations between survival and initial weight or between survival and BW due to high standard errors.

4.4 Genetic parameters and selection response for growth

In this study, we found high h^2 estimates for body weight with a c^2 of around 10%. The h^2 estimates in this study were at the high end of earlier estimates in ponds receiving supplementary feeds (e.g. Velasco et al., 1995; Ponzoni et al., 2005). Eknath et al. (1995) estimated heritability in fertilized ponds receiving no supplementary feeds and obtained a lower h^2 estimate (0.20). Generally, literature indicates widely varying h^2 estimates (0.04 to 0.60, Tave and Smitherman 1980; Eknath et al., 1993; Eknath et al., 1998; Bolivar and Newkirk, 2002; Ponzoni et al., 2005; Rutten et al., 2005a, b) and considerable c^2 (Ponzoni et al., 2005; Rutten et al., 2005a, b) for body weight of mature Nile tilapia in different systems. Although we found higher c^2 in early growth of Nile tilapia in fertilized ponds (Charo-Karisa et al., in press), the c^2 may have reduced with age. The high heritability and low common environmental effects recorded under the rearing conditions in this study indicate sufficient additive genetic effects for selection in low input.

We found a substantial selection response for growth, which was higher than in earlier studies. Earlier studies indicated average genetic gains of between 8.4 –17% per generation in ponds, cages and tanks receiving pelleted feeds (Eknath et al., 1998; Bolivar and Newkirk, 2002; Ponzoni et al., 2005). However, response in the present study may have been over-estimated due to the confounding of genetic and environmental effects. The actual or "realized" phenotypic change will correspond to the expected response to selection only when the environmental effects on the parental and progeny generation are identical (Gall et

al., 1993). The differences between the observed and expected increase BW in this study indicate that the environment in G_2 was better than in G_0 and G_1. Furthermore, G_2 were reared for 25-29 days more than the previous generations. To separate year-effects from genetic effects, it was planned to produce progeny of a number of sires in more than one generation. However, due to fish mortality or tag loss before the next spawning season, this could not be realized. If sires had been used in more than one generation, there would have been genetic links across years which would have resolved the confounding of genetic and environmental effects in the current experiment.

We have compared the selection differential within a generation with the realized change over years. The latter change, however, is the combined effect of selection differential and environmental changes. Results suggest that selection has been successful. To further support that, we have estimated the difference in BW between offspring of the 10 sires with the highest EBV and the average of the offspring from all sires. In G_0, the average EBV of all selected sires was 24.2 g higher than the average of all selected sires in G_0. Based on this, the G_1 offspring of all the best 10 sires are expected to have 12.1 g higher BW assuming average group of dams. The realized difference in G_1 was 9 g or 74.4% of the expected value. The G_2 offspring of the 10 best G1 sires were expected to have 5.2 g higher BW, while the realized difference was 7.8 g or 150% of the expected response. Assuming a four month over-wintering period within which there was negligible growth, the 25 day difference between G_1 and G_2 would have increased the BW of G_2 by 16.4 g, or 38.8% of the realized increase in BW from G_1 to G_2. This clearly demonstrates that the genetic differences between sires result in the expected differences in their progeny.

Restrictions on inbreeding to limit its negative effects need to be considered when implementing a selective breeding program in fish (Gjerde et al., 1996). According to Bijma (2000), the acceptable level of in-breeding, as determined primarily by the extent of inbreeding depression on fitness, is between 0.5% and 1% per generation. In the present study, the inbreeding coefficient after two generations of selection was 0.012. This indicates that the observed response in this study was not at the expense of fitness of the population. It should be noted that with more generations of selection, the inbreeding coefficient is likely to increase. Considering the consistent increase in body weight across generations and the high

heritability estimates, we can conclude that there are good prospects for selection of Nile tilapia for growth in low-input conditions.

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Chapter 6

Phenotypic and genetic parameters for body measurements, reproductive traits and gut length of Nile tilapia *(Oreochromis niloticus)* selected for growth in low-input earthen ponds

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Abstract

This study presents estimates of phenotypic and genetic parameters for body size measurements, reproductive traits, and gut length for Nile tilapia (*Oreochromis niloticus*) selected for growth in fertilized earthen ponds for two generations. Throughout the experiment, ponds were fertilized daily with 50 kg dry matter, (dm) / ha chicken manure. No supplementary feeds were added. For the analysis, 6429 fully pedigreed experimental fish from G0, G1 and G2 were used. Generations were discrete and therefore parameters were estimated separately for each year. Heritability estimates for body measurements were high and ranged from 0.4-0.6 for standard length to 0.69-0.79 for head length. Phenotypic correlations between body weight and body measurements ranged from 0.64 to 0.89. Genetic correlations were close to unity. The heritability estimate for maturity at harvest was 0.13. Heritabilities for carcass traits were estimated from G_1 only and were 0.16 for gutted weight and 0.06 for dressing percentage. Phenotypic correlation between body weight and gutted weight was 0.84 and the genetic correlation was 0.20. Gut length increased with selection for body weight. Heritability estimate for gut length was 0.22. Moreover, gut length and body weight were genetically highly correlated. These results indicate that tilapia selected for growth on a herbivorous diet may develop longer guts as a mechanism for increasing absorptive capacity through increase of surface area and hence more efficient nutrient absorption.

Keywords: Nile tilapia; *Oreochromis niloticus*; Phenotypic correlations; genetic correlations; reproductive traits; body measurements; gut length

1.0 Introduction

The breeding objective of most fish selective breeding programs is enhanced growth rate which leads to more efficient fish production (Gjedrem, 1997). A number of selective breeding programs have been initiated to improve the growth of *O. niloticus* in ponds receiving supplementary feed (Hulata et al., 1986; Eknath et al., 1993; Bensten et al., 1998). These selection programs have typically been carried out in relatively favorable environments receiving supplementary feed. However, there are reports that the gains of selection in Nile tilapia were lost when selected breeds were tested in less favorable environments (Macaranas et al., 1997). This could indicate that the expression of body weight in different

environments, i.e. low and high-input culture conditions, is influenced partly by a different set of genes.

In an earlier paper (Charo-Karisa et al., in prep), we reported phenotypic and genetic parameters for growth and survival from an experiment in which Nile tilapia had been selected for body weight during two generations in fertilized ponds with no supplementary feed. Heritabilities for body weight were between 0.4 and 0.6, and for survival between 0.03 and 0.14. We also observed a considerable selection response for growth. In the present study, we report on the analysis of body measurements, carcass traits and reproductive traits collected during this selection experiment for improved growth of *O. niloticus*. Knowledge of the correlated changes that occur in other traits due to selection for growth is important for the design of an efficient genetic improvement program (Falconer and Mackay, 1996). For Nile tilapia, phenotypic and genetic correlations have been estimated between body weight, body measurements and fillet weight (Rutten et al., 2004; 2005) and between body weight and reproductive traits (Kronert et al., 1989). These authors carried out experiments in indoor recirculation systems and under conditions with high protein pellets, respectively. Phenotypic and genetic correlations between traits in Nile tilapia reared in fertilized ponds are lacking.

2.0 Materials and Methods

2.1 Background information

The experiment was carried out at the Regional Center for Africa and West Asia of the World Fish Center, Abbassa, Egypt. Fish used for this study were the grandparental population (G_0) produced in 2002, the G_1 generation produced in 2003, and G_2 generation produced in 2004. G_1 and G_2 were the first and second generations of selection respectively.

2.2 The founder population and production of G_0

The founder population (i.e. parents of the G_0 population) was produced in spring of 2000 in a full diallel mating design among local Egyptian strains namely Maryout, Zawia, Abbasa and Aswan (Rezk et al., 2002; 2004). Eighty sires and 105 dams, selected at random from among the founder stock, were subsequently used to produce the G_0. Each sire was mated to two dams and each dam mated to only one sire, thus generating full and half sib groups. Fry were raised in 2 x 1 x 1 m hapas suspended in concrete tanks and were fed twice daily with

40% protein supplements, initially in the form of powder and later as pellets. Initial feeding rate was 20% of body weight, which was gradually reduced to 5% body weight at tagging size (i.e. mean wet weight of 2g).

2.3 Production of G_1 and G_2

The first and second generations of selection, G_1 and G_2, were produced in 2 x 3 x 1 m hapas suspended in fertilized ponds. Each sire was mated to two dams as in G_0. Fifty sires and 87 dams were used to produce generation G_1, while for generation G_2, 54 sires and 104 dams were used. At first, each sire was kept in a single hapa with two dams. Twice a week, hapas were checked for occurrence of spawning. Spawning was assumed to have occurred when the dam had eggs or yolk-sac fry in her mouth. The un-spawned dam and the sire were both transferred to an adjacent hapa thus producing the paternal half-sibs. To prevent multiple spawning, the male was removed immediately after spawning occurred. When swim-up fry were sighted in both hapas, the females were also removed. Two to three weeks later, the number of swim-up fry in each hapa was reduced to 80 individuals. In contrast to the G_0, the G_1 and G_2 fry were given no supplementary feeds and were reared in hapas suspended in the earthen ponds. To boost natural pond productivity, ponds with hapas containing fry were fertilized daily with chicken manure at the rate of 50 kg dry matter, dm /ha.

2.4 Grow-out and pond management (G_0, G_1 and G_2)

As soon as a family reached suitable tagging size, 24 randomly chosen fry from each full-sib family were individually tagged with Floy® tags and returned into the hapas until stocking. Fry were between 31-96 days old at stocking. Each family of fry was randomly divided into two groups which were then stocked in two 1000 m^2 fertilized earthen ponds for grow-out. Ponds were daily supplied with dry chicken manure from layer and broiler farms at the rate of 50-kg dry matter (dm)/ha. This fertilization rate corresponds to 0.3 kg nitrogen ha^{-1} day^{-1} which is enough to support yields of 4.3 kg fish BW ha^{-1} day^{-1} (calculated from Knud-Hansen et al., 1991). Fish that died within the first week of stocking were, when possible, replaced with individuals from the same family. After approximately 8 months of growth, which included 3-4 months of over-wintering, fish were harvested by seine netting.

2.5 Trait measurements

Before measurements were taken, fish were anaesthetized with MS222 (3-aminobenzoic acid ethyl ester) to avoid handling stress. Each fish was measured for body weight (BW). Breeding values were estimated from an animal model (Model 1 below). These estimates were used to select 100 males and 200 females as potential brooders for the next generation. After selection of brooders, the following body traits were taken on fish which were not earmarked for breeding: maximum body depth (BD), body thickness (BT), head width (HW), and head length (HL). Body measurements were taken by six persons using vernier calipers. The positions on which the body measurements were taken are shown in Figure 1.

After recording of body measurements, the fish were dissected; guts removed and gutted body weight recorded. Because of high mortality of fish after harvest, only a few fish were gutted in G_0 and G_2. Guts were fixed in 10% formalin for later measurement of gut length. The length of gut (from the stomach to anus) was determined by extending the guts over a meter rule. Gonads were removed and placed between bloating papers to remove excess fluid and weighed. They were then checked macroscopically for determination of maturity stage. Sexual maturity was scored according to Legendre and Ecoutin (1989). Stage 1 refers to immature females (translucent and elongated ovaries with no oocytes); stage 2 refers to females beginning maturation (few small cream coloured oocytes); stage 3 refers to maturing females (light yellow ovaries with numerous distinct small and large oocytes with cytoplasmic vacuoles). Females at stage 4 are ready to spawn (large bright yellow pear-shaped or oval oocytes, with few smaller oocytes), stage 5 are ripe females (predominantly dull yellow large oocytes fully yolked), and stage 6 are spent or post-spawning females (shrunken and reddish ovaries with resorbed oocytes). For males, three maturity stages were scored as follows: Stage 1 as immature (thread-like), stage 2 as maturing (absence of intra-testicular sperm) and stage 3 as mature (presence of intra-testicular sperm). Female gonads at advanced vitellogenesis (stage 4 and 5) were fixed in formalin for determination of fecundity. Absolute fecundity was estimated by counting the number eggs in 0.5 g of the ovaries and multiplying with the total ovary weight.

The weight of visceral organs was determined as the difference between whole body weight and the sum of gutted body and gonad weight. The following traits were calculated for each

fish: Dressing percentage (D%) = gutted body weight * 100/BW; Gut length index (GLI) = gut length/ standard length; and Visceral index = Visceral weight * 100/ BW. Gonadosomatic index (GSI) was determined as = gonad weight * 100/ BW.

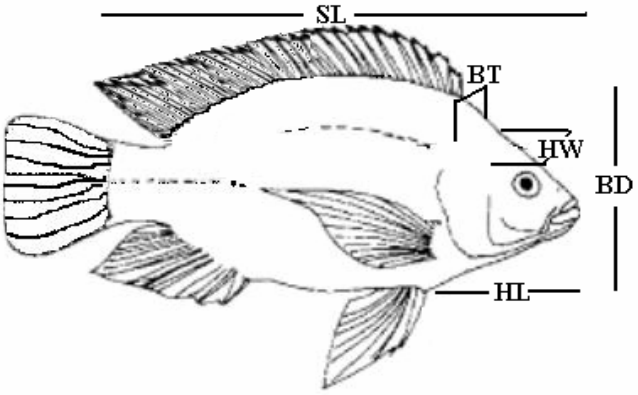

Figure 1. Body measurements taken on each fish: total length (TL), standard length (SL), body depth (BD), body thickness (BT), head width (HW) and head length (HL)

2.6 Data Analysis

The means procedure of SAS (1989) statistical program was used to generate descriptive statistics of the data. Because the generations were discrete, i.e. sires and dams were used only one generation, data from each generation (G_0, G_1 and G_2) were analyzed separately. A complete pedigree of the experimental fish from G_0 onwards was available and was used in each analysis. The basic animal model used was the same model used in Charo-Karisa et al. (in prep):

$$Y_{ijkl} = \mu + PND_i + SEX_j + \beta_1 * AGE_{ijkl} + \beta_2 * log\ (INWT_{ijkl}) + d_k + u_l + e_{ijkl} \ldots. \text{(Model 1)}$$

Where Y_{ijkl} is dependent trait of interest for individual *ijkl* at harvest; μ is overall mean; PND_i is fixed effect of pond (i = 1, 2); SEX_j is the fixed effect of sex (j = 1, 2); β_1 = regression coefficient of the trait on age at harvest; AGE_{ijkl} = co-variable age at harvest of individual *ijkl*; β_2 is regression coefficient of the trait on natural logarithm of initial body weight; $log(INWT_{ijkl})$ is a co-variable of the natural logarithm of initial body weight of individual *ijkl* (the logarithm of initial body weight was used to correct for size differences during the hapa rearing stage); u_l = random additive genetic effect of the lth individual; d_k is a common

environmental/ full-sib effect which included a quarter of dominance effects, and environmental effect of common hapa rearing; and e_{ijkl} = random residual effect associated with an individual.

Heritabilities were estimated using a bivariate setting of Model 1. In the bivariate analysis, the first trait was always body weight, the trait of selection, which was available for all animals. The second trait was measured only on the individuals that not selected as brooders, and the heritability was estimated for this trait. In the bivariate analysis, body measurements were also corrected for the fixed effect of slaughter day. The genetic and phenotypic correlations between traits were estimated from a trivariate setting of the model. In the trivariate model, the first trait was always body weight and the second and third traits were the traits for which correlations were being estimated. As in the bivariate analysis, traits that were measured on the slaughtered individuals were also corrected for the effect of day of slaughter.

Gonad weight and GSI were analyzed separately for each sex. For the estimation of the heritability, the maturity stages were reclassified into two groups, either mature or immature. Female fish at stages 1, 2 or 3 were considered immature and were coded as 0, while fish at stages 4, 5 or 6 were considered mature and were coded as 1. Males at stage 1(immature) were coded as 0 while stages 2 and 3 were coded as 1. Variance components were estimated from the G_1 data set, using a model which included maturity stage as a fixed effect and animal and dam as random effects.

3.0 Results

3.1 Descriptive statistics

The descriptive statistics of body measurements, reproductive traits and gut length are shown in Table 1. Body measurements were taken on 522 fish in G_0, 804 fish in G_1 (after brooders were selected) and 1207 fish (all harvested fish with tags) in G_2. The lower number of fish measured for some traits were due to fish mortalities, and spoilt samples. Mean age at harvest was 289, 293 and 318 days in G_0, G_1, and G_2 respectively. Generally, fish increased in size across generations. The phenotypic means of body weight increased from 67.8 g in G_0 to 129 g in G_2. Standard length increased from 12.0 cm in G_0 to 14.8 cm in G_2; body depth increased

from 41.4 mm in G_0 to 57.0 mm in G_2, and body thickness increased from 18.9 mm in G_0 to 28.7 mm in G_2. Head width increased from 22.6 mm in G_0 to 26.4 mm in G_2, and head length from 40.8 mm in G_0 to 49.4 mm in G_2. The phenotypic mean gutted body weight was 41g in G_0, and 117.7 g in G_2. Dressing percentage varied only a little across generations (86.5% in G_0, 89.6% in G_1, and 86.9% in G_2) and had the lowest coefficient of variation (mean = 4.3%). Gut length increased from 43.8 cm in G_0, to 80.4 cm in G_2; the corresponding gut length indices were 3.0, 3.2 and 4.2.

Table 1. Means ($\bar{x}$), standard deviations (σ), coefficients of variation, % (CV) of age at harvest, initial weight, body weight at harvest, body measurements, gutted weight, visceral weight, dressing (%), reproductive traits, and gut length measurements in G_0, G_1, and G_2.

	G_0				G_1				G_2			
Trait	n	$\bar{x}$	σ	CV	n	$\bar{x}$	σ	CV	n	$\bar{x}$	σ	CV
Age (day)	1225	289.0	22.9	7.9	1288	293.3	8.9	3.0	1207	318.2	12.7	4.0
Initial weight (g)	2122	2.4	2.1	87.9	2101	3.8	1.8	46.7	2206	4.3	3.2	74.2
Body weight (g)	1225	67.8	37.7	55.6	1288	86.0	32.9	38.2	1207	129.0	41.0	31.8
Standard length (cm)	522	12.0	1.6	13.5	1288	13.3	1.8	13.2	1207	14.8	1.6	11.0
Body depth (mm)	522	41.4	6.0	14.5	804	46.8	5.1	11.0	1207	57.0	6.9	12.1
Body thickness (mm)	522	18.9	2.9	15.6	804	22.1	2.8	12.8	1207	28.7	3.3	11.5
Head width (mm)	522	22.6	3.0	13.2	804	25.3	2.8	10.9	1207	26.4	3.0	11.4
Head length (mm)	522	40.8	5.3	12.9	804	43.6	4.1	9.4	1207	49.4	5.1	10.3
Gutted wt (g)	442	41.0	17.3	42.3	750	63.6	20.5	32.3	262	117.7	32.1	27.2
Dressing (%)	442	86.5	4.1	4.7	750	89.6	2.8	3.1	262	86.9	4.4	5.1
Gut length (cm)	130	43.8	9.2	20.9	693	49.7	12.8	25.7	262	80.4	16.3	20.2
Gut length index	130	3.0	0.6	18.7	693	3.2	0.8	24.4	262	4.2	0.8	18.6
Visceral wt (g)	435	5.8	2.9	50.5	747	9.4	3.0	32.0	253	13.9	5.6	40.2
Visceral index	435	14.6	5.1	34.6	747	8.4	2.3	27.4	253	11.9	4.4	37.0
Maturity ♂	247	85.0	–	–	420	86.5	–	–	169	82.6	–	–
Maturity ♀	188	39.5	–	–	329	64.3	–	–	85	86.0	–	–
Gonad wt, ♂ (g)	247	0.2	0.2	115.8	420	0.6	0.5	89.0	169	0.8	1.0	127.3
Gonad wt, ♀ (g)	188	0.5	0.8	151.1	329	2.2	1.2	54.5	85	3.8	2.0	52.8
Fecundity	94	506.0	312.5	61.7	150	740.0	367.0	49.6	40	1323.0	620.0	46.9
Eggs/kg BW	94	6665.0	5200.5	78.0	150	12138.0	3695.9	30.4	40	10913.0	4675.9	42.8
GSI ♂	247	0.4	0.4	97.4	419	0.7	0.7	100.0	169	0.5	0.7	140.0
GSI ♀	188	1.5	1.8	117.7	329	3.5	1.7	48.5	85	3.3	1.6	48.0

Fecundity was measured on 94 females in G_0, 150 females in G_1 and 40 females in G_2. Mean fecundity increased from 506 eggs per female in G_0 to 1323 eggs in G_2, while the number of eggs/kg body weight increased from 6665 in G_0 to 10913 in G_2. The proportions of fish at

different maturity stages are shown in Figure 2. Among both males and females, the proportion of immature fish (stage 1) at harvest decreased to 0% by G2. The proportion of ripe female fish (stage 4 and 5) increased from 20.2% in G_0 to 55.8% in G_2.

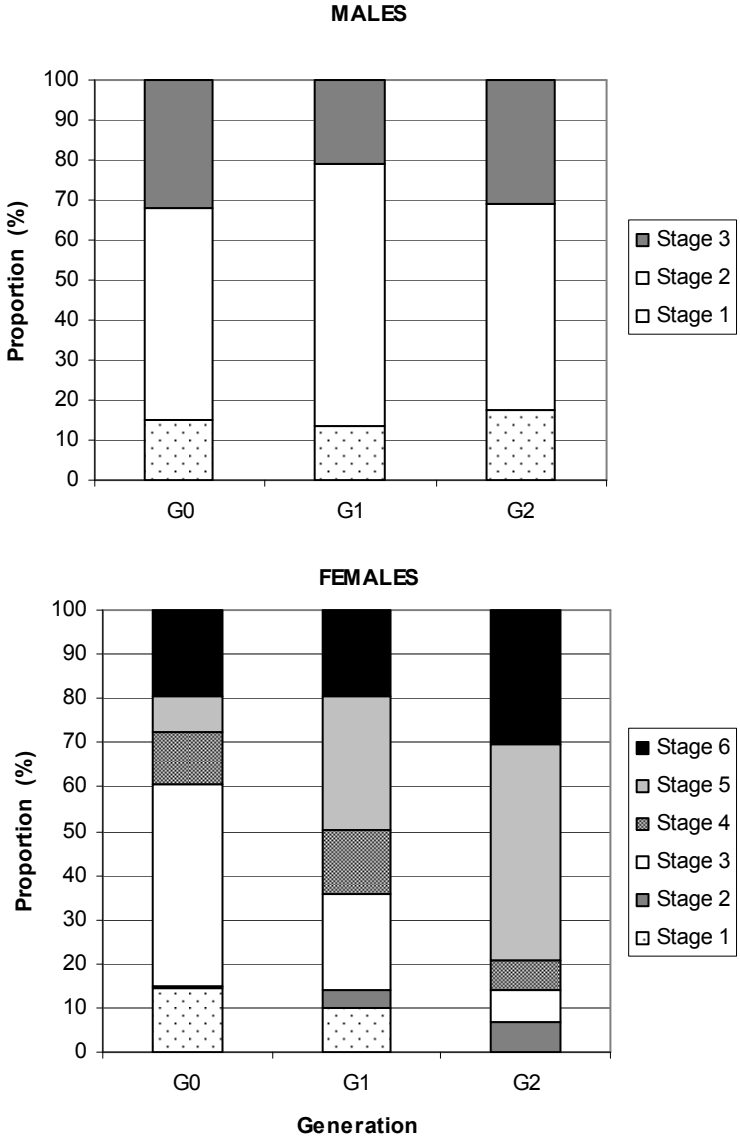

Figure 2. Proportion of male and female Nile tilapia at different maturity stages at harvest in generation G_0, G_1, and G_2.

3.2 Additive genetic and common environmental/full-sib effects

The heritability estimates for body measurements were between 0.40 and 0.79 (Table 2). In general, with the exception of head width, heritabilities among body measurements were higher in G_2 than in G_0 and G_1. Because of low fish numbers caused by high mortality after harvest, it was only possible to estimate genetic parameters for carcass, gut length and

reproductive traits in G_1 (Table 3). The heritability estimates for both gutted weight was 0.16, and for dressing (%) was 0.06. The heritability estimates for gut length was 0.22 and that for gut length index was 0.09. The heritability estimates for visceral weight and visceral index were 0.44 and 0.03, respectively. The heritability estimate for male gonad weight was higher (0.25) than for female gonad weight (0.18) whereas the heritability for female GSI was higher (0.25) than for male GSI (0.03). There was generally low common environmental/ full sib effect (c^2) for body traits, and reproductive traits (Table 2 and 3).

Table 2. Estimates of total phenotypic variance (σ^2_P), heritability (h^2) and common environmental/ full-sib effects (c^2) with standard errors (in brackets) for body weight at harvest (BW), standard length (SL), body depth (BD), body thickness (BT), head width (HW) and head length (HL) in G_0, G_1, and G_2.

	G_0			G_1			G_2		
Trait	σ^2_P	h^2	c^2	σ^2_P	h^2	c^2	σ^2_P	h^2	c^2
BW*	782.2	0.60 (0.08)	0.09 (0.07)	510.0	0.38 (0.12)	0.11 (0.07)	794.0	0.51 (0.19)	0.08 (0.07)
SL	4.0	0.59 (0.08)	0.01 (0.01)	1.6	0.40 (0.08)	0.03 (0.01)	1.4	0.60 (0.09)	0.01 (0.01)
BD	59.2	0.64 (0.08)	0.00 (0.00)	24.7	0.65 (0.03)	0.00 (0.00)	22.4	0.73 (0.10)	0.02 (0.01)
BT	14.4	0.60 (0.08)	0.00 (0.00)	6.9	0.53 (0.09)	0.00 (0.00)	5.7	0.62 (0.09)	0.02 (0.01)
HW	14.9	0.64 (0.08)	0.00 (0.00)	6.4	0.64 (0.09)	0.01 (0.01)	4.3	0.56 (0.09)	0.03 (0.01)
HL	43.6	0.66 (0.08)	0.00 (0.00)	17.7	0.69 (0.09)	0.00 (0.00)	13.6	0.79 (0.09)	0.01 (0.01)

* Also reported in Charo-Karisa et al., in prep.

3.3 Phenotypic and genetic correlations

Phenotypic and genetic correlations between the selection trait, body weight, and other traits are presented in Table 4. Only the G_1 dataset was used for estimation of correlations between traits. The phenotypic correlations between body weight and body measurements were between 0.64 and 0.89 and the genetic correlations were between 0.95 and 0.99. The phenotypic correlation between body weight and gutted weight was 0.84, while the genetic correlation was 0.20. On the other hand, the phenotypic correlation between body weight and dressing percentage was 0.23 while the genetic correlation was 0.65 but with high standard error. The phenotypic and genetic correlations between body weight and gut length were 0.29 and 0.82 respectively. Except for the genetic correlation between body weight and gonad weight of females, other correlations between body weight and reproductive traits were low. A full table of phenotypic and genetic correlations between traits is presented in Appendix 1.

Table 3. Estimates of total phenotypic variance (σ^2_P), heritability (h^2) and common environment effects/ full-sib effect (c^2) with standard errors (in brackets) of carcass and reproductive traits in G_1

Trait	σ^2_P	h^2	c^2
Gutted weight	168.9	0.16 (0.11)	0.06 (0.05)
Dressing (%)	5.2	0.06 (0.07)	0.04 (0.08)
Gut length	156.2	0.22 (0.07)	0.00 (0.00)
Gut length index	0.5	0.09 (0.05)	0.00 (0.00)
Visceral weight	4.4	0.44 (0.09)	0.01 (0.01)
Visceral index	22.2	0.03 (0.07)	0.01 (0.03)
Maturity	0.2	0.13 (0.05)	0.00 (0.00)
Gonad weight ♂	0.2	0.25 (0.09)	0.00 (0.00)
Gonad weight ♀	1.1	0.18 (0.10)	0.02 (0.04)
GSI ♂	0.5	0.03 (0.05)	0.00 (0.00)
GSI ♀	2.5	0.25 (0.10)	0.00 (0.00)

Table 4. Phenotypic and genetic correlations between body weight and body measurements, carcass traits and reproductive traits from G_1

Trait	Phenotypic	Genetic
Standard length	0.64 (0.04)	0.95 (0.03)
Body depth	0.86 (0.03)	0.99 (0.01)
Body thickness	0.82 (0.03)	0.99 (0.01)
Head width	0.89 (0.03)	0.99 (0.01)
Head length	0.78 (0.05)	0.98 (0.01)
Gutted body weight	0.84 (0.08)	0.20 (0.07)
Dressing (%)	0.23 (0.04)	0.65 (0.47)
Gut length	0.29 (0.09)	0.82 (0.09)
Gut length index	0.03 (0.09)	0.22 (0.28)
Maturity	0.26 (0.10)	0.18 (0.24)
Gonad weight ♂	0.13 (0.01)	0.05 (0.01)
Gonad weight ♀	0.33 (0.17)	0.68 (0.25)
GSI ♂	0.06 (0.01)	0.01 (0.00)
GSI ♀	0.20 (0.12)	0.27 (0.17)

4.0 Discussion

In this study, we estimated heritability for traits in Nile tilapia reared in fertilized ponds with no supplementary feeding. We found high heritability estimates for body measurements with low common environmental effects. In an earlier study in earthen ponds, Velasco et al. (1995) found similarly high heritability estimates for body depth and body length. In contrast, Rutten et al. (2005) found lower estimates for body depth, body thickness and body length in recirculation systems. However, the low common environmental effects found in the present study agree well with Rutten et al. (2005). Although negligibly low, the common

environmental/full-sib effects for body measurements in the present study tended to be slightly higher in later generations.

The high correlations between body weight and body measurements in the present study agree with findings of Rutten et al. (2004; 2005). In the present study, heritability estimates were higher for body measurements than for body weight. In contrast, the heritability estimates for body measurements in recirculation systems (Rutten et al., 2005) were either similar or lower than for body weight. When the correlated trait has a substantially higher heritability than the primary selection trait and the genetic correlation between the traits is high as in this study, indirect selection may be used (Falconer and Mackay, 1996). Our findings suggest that selection for body measurements is a good alternative for measuring weight. This may be the only option in field conditions where (electronic) weighing balances are not available.

Compared to recirculation systems, the Nile tilapia harvested in fertilized ponds are on average smaller and are usually not filleted. The fish farmer is therefore paid either for whole or gutted body weight; gutted weight being a direct measure of how much meat the consumer can get. In this study, the phenotypic correlation between body weight and gutted body weight was high but the genetic correlation was low. Although the genetic correlation between body weight and gutted body weight was low, we recorded high phenotypic means for gutted weight in G_2 which was higher than the corresponding increase in body weight. Although the increase in gutted weight may also be due to environmental influences given the close resemblance with the increase in body weight, one would expect that gutted body weight would have a higher genetic correlation with body weight than is reported here. The low genetic correlation between gutted body weight and body weight is therefore doubtful. The consistent increase in gutted body weight across generations suggests that selection for body weight will also improve gutted body weight. Similar to the present study, Gjerde and Gjedrem (1984) obtained low heritability for dressing percentage in Atlantic salmon and rainbow trout. Also, Rutten et al. (2005), working with Nile tilapia in recirculation systems, concluded that no further response in fillet weight can be achieved by including dressing percentage in a selection index with body weight. Although the genetic correlation between

body weight and dressing percentage was with large standard errors, these results suggest that dressing percentage may be improved by selection for body weight at harvest alone.

The phenotypic and genetic correlations between body weight and gonad weight, body weight, GSI, and maturity obtained in this study were comparable to earlier studies in *O. niloticus* by Kronert et al. (1989). Similar to the present study, Eknath et al. (1995) found heritability for maturity at spawning of 0.15. The low genetic correlation between maturity and body weight found in this study is consistent with studies in other fish species (Gjerde, 1986; Gjerde et al., 1994). Although excessive reproduction in culture ponds reduces the growth of adult fish, low fecundity of tilapia can hinder the production of adequate numbers of fingerlings for grow-out (Coward and Bromage, 2000). Given the considerable heritability, the reproductive capacity of females and maturity at an earlier age can be improved by selection when necessary. Longalong et al. (1999) pointed out that selection for lower frequency of early maturing females in Nile tilapia may result in poorer growth and that selection for improved growth may increase the frequency of early maturing females. The low genetic correlation between maturity and body weight at harvest in the present study indicate that it is possible to simultaneously select for late maturity and fast growth. The fecundity and the number of eggs/kg body weight (10912 to 12138) in this study were consistent with earlier studies (Smitherman et al., 1988; Duponchelle et al., 1998) indicating that selection in low-input ponds did not reduce fecundity. We did not have difficulties when reproducing the selected individuals. It should be emphasized however, that because of high plasticity of reproductive traits in Nile tilapia (Pullin, 1982; Kronert et al., 1986; Duponchelle et al., 1998; Duponchelle and Legendre, 2001) routine monitoring of reproductive parameters should be done in tilapia breeding programs.

In this study, our fish were grown on a periphyton/planktonic diet induced by manure fertilization. Herbivorous fishes are known to have longer intestines than carnivorous or omnivorous fishes because they live on food requiring greater digestive processing (Nagase, 1964; Smith, 1989). Therefore, we expected that selection for growth would lead to tilapia with longer relative intestine lengths. In the present study, we found that larger fish tended to have higher gut length to size ratios. In contrast, Abdel-Tawwab and El-Marakby (2004) found no differences in gut lengths of Nile tilapia between 25-125 g body weights. It has

been suggested that fish size may be genetically correlated with the efficiency for absorption of nutrients (Gjedrem and Thodesen (2005), and that growth rates of animals are fundamentally dependent upon the systems where digestive and absorptive processes occur (Zimmerman et al., 2005). In the present study, we found substantial additive genetic variance for gut length and that gut length increased with selection for body weight. Gut length and body weight were also highly genetically correlated. According to Ferraris and Ahearn (1984), guts of herbivorous fish are long and appear structurally uniform from stomach to rectum. However, Sklan et al., (2004) found that tilapia fed fish meal diets did not have uniform structure and that the sites of nutrient absorption were altered. The intestine of hybrid tilapia *O. niloticus* X *O. aureus* comprises a series of nested loops, which are lined with villi on the luminal face, considerably enlarging the absorptive area (Sklan et al., 2004). This suggests that having longer guts may be a mechanism for increasing absorptive capacity through increase of surface area and hence more efficient nutrient absorption especially for tilapia fed on a diet of periphyton and phytoplankton.

In this study Nile tilapia were selected for growth in low-input fertilized ponds. Previously, we reported high heritability estimates and response to growth (Charo-Karisa et al., in prep). The present findings on the correlated responses in body measurements and gut length indicate that fish selected for growth in low input environments might become very different from fish selected for growth on a diet of high protein pellets. Because of the diversity of Nile tilapia fish farming systems it will be crucial to know how fish selected in high or low input environments will perform in these different farming systems.

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Appendix 1

Estimated phenotypic (above diagonal) and genetic correlations (below diagonal) between traits in 300 day old Nile tilapia in G_1. Standard errors are in (superscripts). BW= whole body weight, SL = standard length, BD= body depth, BT = body thickness (width), HW = head width, HL= head length, GBW = gutted body weight, GL = gut length, D (%) = dressing percentage, and GLI = gut length index.

Trait	BW	SL	BD	BT	HW	HL	GBW	GL	D%	GLI
BW		0.64 (0.04)	0.86 (0.03)	0.82 (0.03)	0.89 (0.03)	0.78 (0.05)	0.84 (0.08)	0.29 (0.09)	0.23 (0.04)	0.03 (0.09)
SL	0.95 (0.03)		0.51 (0.07)	0.43 (0.07)	0.50 (0.07)	0.47 (0.08)	0.59 (0.03)	0.27 (0.08)	0.13 (0.07)	0.09 (0.08)
BD	0.99 (0.01)	0.92 (0.04)		0.73 (0.05)	0.80 (0.04)	0.87 (0.01)	0.72 (0.06)	0.46 (0.09)	0.16 (0.10)	0.22 (0.11)
BT	0.99 (0.01)	0.90 (0.05)	0.98 (0.01)		0.81 (0.03)	0.65 (0.06)	0.59 (0.03)	0.47 (0.08)	0.01 (0.08)	0.28 (0.08)
HW	0.99 (0.01)	0.94 (0.03)	0.98 (0.01)	0.99 (0.01)		0.74 (0.06)	0.71 (0.05)	0.50 (0.08)	0.16 (0.10)	-0.03 (0.01
HL	0.98 (0.01)	0.95 (0.03)	0.72 (0.08)	0.97 (0.01)	0.98 (0.01)		0.67 (0.07)	0.40 (0.10)	0.21 (0.11)	0.19 (0.11)
GBW	0.20 (0.07)	0.35 (0.04)	0.26 (0.03)	0.30 (0.03)	0.27 (0.03)	0.20 (0.02)		0.37 (0.04)	0.24 (0.01)	-0.03 (0.01)
GL	0.82 (0.09)	0.86 (0.06)	0.95 (0.03)	0.92 (0.04)	0.96 (0.03)	0.92 (0.03)	0.16 (0.03)		0.06 (0.07)	0.45 (0.02)
D%	0.65 (0.47)	-0.17 (0.23)	-0.23 (0.25)	-0.19 (0.23)	-0.27 (0.23)	-0.28 (0.24)	0.32 (0.13)	-0.59 (0.47)		0.02 (0.07)
GLI	0.22 (0.28)	0.78 (0.10)	0.89 (0.09)	0.85 (0.07)	0.56 (0.00)	0.85 (0.09)	-0.04 (0.01)	0.04 (0.01)	-0.54 (0.21)	

Chapter 7

Genotype by environment interaction in two lines of Nile tilapia (*Oreochromis niloticus* L.) divergently selected for growth in different pond environments

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Abstract

This study was designed to determine the extent of genotype by environment interaction in Nile tilapia and to support the decision on the best environment for selection of Nile tilapia for a wide range of farming conditions. We used two lines of Nile tilapia that had been divergently selected for two generations in two earthen ponds environments. One line was selected in earthen ponds that were fertilized daily with chicken manure but no supplementary feeding (low line). The other line was selected in ponds where fish received 25% protein pellets (high line). The 2 lines were tested in five test environments: 40% protein pellets feed (P200), 25% protein pellets (P100), 16% protein pellets (P50), 50 kg/ha chicken manure (M100) and 25 kg/ha chicken manure (M50). Nitrogen input was similar in P50 and M50, and in P100 and M100 treatments respectively. Survival from stocking to harvest ranged from 70-75 % in the high line and from 62 to 76% in the low line. Analyses revealed significant differences in growth performances of the two lines across test environments and the high line performed better in most test environments. The phenotypic mean body weight at harvest was highest for test environment P200 (123.4 g in the low line, and 131.7 g in the high line). Lowest phenotypic body weight means were 92.1g (test environment M50) in the low line and 82.4g (test environment P100) in the high line. Further, we found significant evidence for line by test environment interaction. Family by test environment interaction was significant only in the low line. This genotype by environment interaction was related to the interaction of the families to nitrogen and dissolved oxygen in the ponds.

Keywords: genotype by environment interaction; Nile tilapia; *Oreochromis niloticus*; low input environments

1. Introduction

The effect of selection environment on the performance of selected strains over a range of potential production environments is a fundamental breeding question. When genotypes differ in sensitivity to different environmental influences, a genotype by environment interaction (GXE) occurs (Falconer, 1990). In the presence of GXE, the genetic improvement obtained by selection in one environment may not be realized in other environments. Using experimental evidence, Jinks and Connolly (1973, 1975) described the relationship between the selection environment and environmental sensitivity, and concluded that environmental

sensitivity was reduced if selection and environment effects were in opposite directions (antagonistic), and that sensitivity was increased if selection and environment effects were in the same direction (synergistic). Falconer (1990) reviewed a number of experiments and indicated that, in most cases, to increase the mean performance of a genotype over a range of environments, selection should be done upwards in the less favourable environment (antagonistic).

Nile tilapia (*Oreochromis niloticus* L.), is the most important cultured fish species among the tilapiines. Because of high cost of supplementary feeds (Wu et al., 1999), poor farmers especially in sub-Saharan Africa, either grow Nile tilapia using fertilization with organic material alone or with a wide variety of locally available farm resources (Liti et al., 2005) making its farming conditions quite diverse. A number of selective breeding programs to improve the growth of *O. niloticus* have been initiated (e.g. Hulata et al., 1986; Eknath et al., 1993; Bensten et al., 1998; Eknath et al., 1998). These programs have typically been carried out in relatively favourable environments where fish receive supplementary feed. However, there are reports that the gains of selection were lost when selected breeds were tested in less favourable environments (Macaranas et al., 1997). This could indicate that growth in different culture conditions is subject to genotype by environment interaction.

A selective breeding program aimed at producing a breed with improved performance over a wide range of earthen pond environments has been initiated at the WorldFish Center, Abbasa, Egypt, in 2002. Fish from a common germplasm were divergently selected in two environments differing in the level and source of nitrogen input: (i) a favourable environment in which fish were fed on 25% protein pellets by demand feeders and (ii) a less favourable environment in which fish were fed on natural pond food induced by application of chicken manure at low level (50kg dry matter /ha/day). After two generations of selection, the fish from both selection lines were tested in five environments chosen to cover a wide range of the environments used in Africa and other resource poor regions. This paper presents the result of that experiment. The objective of this study was to compare the growth of the selected lines of Nile tilapia in different environments, to estimate the level of GXE in each line, and to determine the environmental sensitivity of the selected lines to support the decision on the best environment for selection of *O. niloticus* for a wide range of resource-

poor environments.

2. Materials and Methods

2.1 Experimental design and test environments

The experiment was carried out in thirty 200 m^2 concrete walled ponds with earthen bottoms. The experimental ponds were arranged in three rows of ten. Ponds were assigned to five test environments in three replicates (for each selection line). The layout of the ponds on-site and assignment of test environments is shown in Figure 1. In each column, each line and test environment was randomly assigned to ponds. Five different test environments differentiated by the level and type of nitrogen sources were used: commercially available 40% protein floating pelleted feed (P200), commercially available 25% protein floating pellets (P100), commercially available 16% protein floating pellets (P50), 50kg dry matter/ha chicken manure (M100) and 25kg dry matter/ha chicken manure (M50). Chicken manure was sourced from broiler and layer farmers near the study area. The fish in pellet-fed ponds were fed to satiation twice (10.00 hr and 15.00hr) per day whereas fish in the manure fertilized ponds depended on natural food which was stimulated by the daily (10.00 hr) administration of chicken manure.

M50 L	P50 H	P100 L
M50 H	M100 H	P200 H
P50 L	P100 H	M100 L
P200 H	P200 L	M100 H
M100 L	M50 H	P100 H
P100 L	M50 L	P50 L
P50 H	P100 L	M50 H
M100 H	P200 H	P200 L
P100 H	P50 L	M50 L
P200 L	M100 L	P50 H

Figure 1. Schematic presentation of experimental pond layout and assignment of test environments to ponds for the low (L) and high-input (H) selection lines (M50= 25kg/ha/day chicken manure; M100= 50kg/ha/day chicken manure; P50= 16% protein pellets; P100= 25% protein pellets; and P200= 40% protein pellets).

2.2 The founder population and production of G_0

The founder population (i.e. parents of the G_0 population) was produced in spring of 2000 in a full diallel mating design among local Egyptian Nile tilapia strains (Rezk et al., 2002; 2004). Eighty sires and 105 dams, selected at random from among the founder stock, were used to produce the G_0. Each sire was mated to two dams and each dam mated to only one sire, thus generating full and half sib groups. Fry were raised in 2 x 1 x 1 m hapas suspended in concrete tanks and were fed twice daily with 40% protein supplements, initially in the form of powder and later as pellets. Initial feeding rate was 20% of body weight, which was gradually reduced to 5% body weight at tagging size (i.e. mean wet weight of 2g). Fry from each family were individually tagged with Floy® tags and returned to the hapas until all families were tagged. Each family was then divided into two groups. One line was reared in a low-input pond environment and the other in a high-input pond environment.

2.2.1 Production of the high line

The first and second generations of selection (G_1 and G_2) were produced in the same way as the G_0 above. After tagging, the fry were communally grown in a 1000 m^2 earthen pond at a density of 2 fish per m^2. In the ponds, fish were fed with 25% protein pellets administered through demand feeders. The grow-out period was approximately 8 months. This period included 3-4 months of over-wintering, which occurred in the pond. After harvesting, fish were ranked based on their estimated breeding values from an animal model. Brooders were selected based on the rank of EBVs, separately for males and females. A hundred males and 200 females were selected as potential brooders for the next generation. We aimed at spawning the best ranking 50 males and 100 females. However, to minimize inbreeding, the number of individuals that were selected from a single full sib or half sib group was restricted allowing fish that were not among the best ranks (up to the rank of 100 for males and 200 for females) to be included as brooders.

2.2.2 Production of the low line

The first and second generations of selection (G_1 and G_2) were spawned and reared in 2 x 3 x 1m hapas suspended in fertilized nursery ponds. No supplementary feeds were given, but the ponds were fertilized daily with chicken manure at a rate of 50kg dry matter (dm)/ ha. After tagging, fry were stocked in two 1000m^2 ponds at a stocking density of 1 fish m^{-2}. As in the

nursery ponds, chicken manure was added daily at 50kg dm/ ha. No supplementary feeds were given throughout the study period. The grow-out period was the same as that for the high line (8 months including 3-4 months of over-wintering). Fish were harvested at the same time as the high line. After harvesting, fish were ranked based on their estimated breeding values from an animal model. Brooders were selected based on the rank of EBVs, separately for males and females. As in the high line the aim was to spawn the best ranking 50 males and 100 females but animals of lower ranking were used.

2.3 Production of experimental fish

Fish used for the genotype by environment interaction experiment were produced from the second generation animals (G_2) of both lines. The experimental fish were obtained from the best 37 sires and 40 dams from the high line, and 32 sires and 58 dams from the low line. Fry from each line were reared in their respective nursing conditions until tagging (from 35 days old). After tagging, each full-sib family, represented by at least 75 fry, was divided into five groups of 15 fry each and assigned to the five different test environments i.e. five fry per family per replicate pond. All ponds were stocked at the same stocking density of 1 fish/ m^2. Due to tag mortalities, not all fish survived to stocking. Equal stocking densities at the start of the experiment were maintained by stocking some untagged fish from the same families. Temperature, dissolved oxygen and pH were measured twice a week in all ponds. Measurements were taken in the morning (6.00-7.00 h) and afternoon (15.00-16.00 h) with a portable DO meter (WTW® model multi 340i meter). After 4 months of growth (August to November), the fish were harvested by seine netting and the body weight of each fish measured.

2.4 Data analysis

Means of body weight for each line and test environment were computed by the means procedure of SAS (1989). Analyses of data were carried out using general linear models (GLM procedure of SAS). To obtain homogeneous residuals a logarithm transformation was used. The present experiment allows studying genotype by environment interaction at two different levels: differences in sensitivity to the environment between individuals from different lines, or differences in environmental sensitivity between individuals from different

families within a line. The following models were used- model 1 across lines and model 2 within lines:

$$Y_{ijkl} = \mu + TE_i + SEX_j + LINE_k + POND_l(LINE_k*TE_i) + \beta_1*AGE_{ijkl} + \beta_2*ln(w)_{ijkl}*\text{line}_k + LINE_k*TE_i + e_{ijkl} \quad \text{(Model 1)}$$

$$Y_{ijklm} = \mu + TE_i + SEX_j + POND_k(TE_i) + \beta_1*AGE_{ijklm} + \beta_2*ln(w)_{ijklm} + FAM_l + FAM_l*TE_i + SEX_j*TE_i + e_{ijklm} \quad \text{(Model 2A)}$$

Where Y_{ijkl} *or* Y_{ijklm} is natural logarithm body weight at harvest for the *l*th or *m*th individual; μ is the overall mean; TE_i is the fixed effect of test environment (i = 1, 2, 3, 4, 5); SEX_j is the fixed effect of sex (j = 1, 2); $LINE_k$ is the effect of the *k*th selection line (k = 1, 2); $POND_l(LINE_k*TE_i)$ is the fixed effect of the *l*th pond nested within line by test environment interaction; $POND_k(TE_i)$ is the fixed effect of the *k*th pond within the *i*th test environment; β_1 is the regression coefficient of natural logarithm of body weight at harvest on age at harvest; AGE_{ijkl} or AGE_{ijklm} is a co-variable of age at harvest of the *l*th or *m*th individual; β_2 is the regression coefficient of natural logarithm of body weight at harvest on natural logarithm of initial body weight; $ln(w)_{ijkl}$ or $ln(w)_{ijklm}$ = a co-variable of the natural logarithm of initial body weight of the *l*th or *m*th individual; FAM_l is the effect of the full-sib family *l*; and e_{ijkl} or e_{ijklm} is random residual effect associated with individual *k* (Model 1) or individual *m* (Model 2). In the high line, age of the fry and full-sib family effect were confounded; consequently this effect could not be estimated. First order interaction of line by test environment or family by test environment and sex by test environment effects were included in both models to check for indication of genotype by environment and sex by environment interactions.

To determine the extent of genotype by environment interactions of families among test environments, we computed Pearson correlation coefficients between the least square means of the family by environment interaction in Model 2A. For given values of genetic correlation and heritabilities, the expected correlation between full sib family means can be derived as:

$$r_{FS-Fam} = \frac{\frac{1}{2}r_A h_1 h_2 + \left[\frac{\frac{1}{2}r_A h_1 h_2}{n}\right]}{\sqrt{\frac{1}{2}h_1^2 + \left[\frac{1-\frac{1}{2}h_1^2}{n}\right]}\sqrt{\frac{1}{2}h_2^2 + \left[\frac{1-\frac{1}{2}h_2^2}{n}\right]}} \quad \text{…equation 1}$$

where r_{FS-Fam} is the correlation of full sib family means, n is the number of full sib family offspring in each environment (assumed to be equal in both environments), h_1^2=the heritability in environment 1, h_2^2 = the heritability in environment 2 and r_A is the genetic correlation between the traits measured in environment 1 and environment 2. The Pearson correlations of full sib family means between test environments were compared to the expected genetic correlations taking 10 as the number of progeny per full-sib in each test environment and assuming a heritability of 0.4 in both environments (based on Charo-Karisa et al., in prep).

Initially, replicate ponds were to receive the same amount of feed or chicken manure. However, in some ponds not all feed was consumed and to maintain suitable water quality, the amount of feed and manure was reduced in these ponds. Similarly, manure fertilization was discontinued when morning dissolved oxygen fell below 1 mg/l and was resumed when the level of dissolved oxygen was above 1 mg/l. The total amount of nitrogen supplied in each pond was determined by taking the average of the actual amount of pellets or manure that was applied to all ponds within a test environment. The amount of nitrogen in the pellets was derived from the relationship: Crude protein = 16% N; the amount of crude protein in the feed was calculated from the percentage protein in each feed. The amount of nitrogen released from the manure was determined graphically from Knud-Hansen et al. (1991).

Because different ponds received differing amounts of nitrogen input and due to differences in dissolved oxygen, we further tested the effect of nitrogen input and dissolved oxygen on fish performance. The aim was to see if nitrogen or dissolved could be used as descriptors of the test environments. This was done using a modified Model 2A:

$$Y_{ijklm} = \mu + SEX_j + TE_i + \beta_1 * AGE_{ijklm} + \beta_2 * ln(w)_{ijklm} + FAM_l + FAM_l * TE_i + SEX_j * TET_i + e_{ijklm}$$

(Model 2B)

where the model terms are as for Model 2A.To determine the effect of nitrogen and dissolved oxygen, the fit (R^2) of Model 2B was compared with the R^2 of two models in which

(i) Test environment (TE) was replaced with either nitrogen or dissolved oxygen, and

(ii) Test environment and the interaction terms were removed i.e. leaving only the effect of sex, log initial weight and family effect. Because the relation between body weight at harvest with nitrogen and dissolved oxygen followed a quadratic trend, quadratic terms of nitrogen and dissolved oxygen and their interaction with family and sex were included in the model.

Table 1. Number of fish at stocking, mean survival (%), and phenotypic mean, standard deviation (SD) and coefficients of variation (CV, %) for initial body weight (at stocking) and body weight at harvest for each selection line in different test environments

			Initial weight (g)			Survival	Body weight at harvest (g)		
LINE	Environment*)	N	Mean	SD	CV	(%)	Mean	SD	CV
Low	M50	630	3.7	1.79	48.4	76.3	92.1	30.5	33.1
	M100	626	3.5	1.76	49.7	69.7	100.6	29.5	29.4
	P50	630	3.6	1.68	46.7	75.0	110.6	38.1	34.4
	P100	624	3.5	1.66	46.7	72.0	99.8	31.5	31.5
	P200	630	3.6	1.83	50.0	62.3	123.4	63.3	51.3
	Pooled	3140	3.6	1.74	48.4	71.1	104.5	40.8	39.0
High	M50	623	1.1	0.50	46.8	73.3	102.3	36.7	35.8
	M100	618	1.1	0.54	49.2	70.0	105.9	32.3	30.5
	P50	624	1.1	0.51	47.7	73.0	92.8	29.5	31.8
	P100	623	1.1	0.48	45.3	74.7	82.4	29.3	35.6
	P200	622	1.1	0.55	50.8	71.3	131.7	52.0	39.5
	Pooled	3110	1.1	0.52	48.0	72.5	103.1	40.3	39.1

*)M50 = 25 kg/ha/day chicken manure; M100 = 50 kg/ha/day chicken manure; P50 = 16% protein pellets; P100 = 25% protein pellets; and P200 = 40% protein pellets.

3. Results

Mean age at stocking was higher for the low line (mean 73 days; range 46-87 days) than the high line (mean 58 days; range 43-73 days). Mean body weight at stocking (initial body weight) was also higher for the low line (3.6 g) than for the high line (1.1 g) (Table 1). The coefficient of variation for initial body weight was similar in both lines and ranged from 46.7 to 50% (mean 48.4%) in the low-line and 45.3 to 50.8% (mean 48%) in the high line. Survival till harvest ranged from 62.3% to 76.3% (mean 71.1%) in the low line and from 70 to 74.7% (mean 71.8%) in the high line. The low survival in the P200 test environment in the low line was mainly due to an unexpected low survival (54.3%) in one of the three replicate ponds. In both lines, phenotypic mean body weight at harvest was highest for test

environment P200 (123.4 g for the low line, and 131.7 g for the high line; Table 1). Lowest growth was found in test environment M50 for the low line and P100 for the high line. The coefficients of variation for body weight at harvest were between 29.4-51.3% in the low line and 30.5-39.5% in the high line. In both lines, test environment P200 tended to have a higher coefficient of variation; being highest in the low line (51.3%).

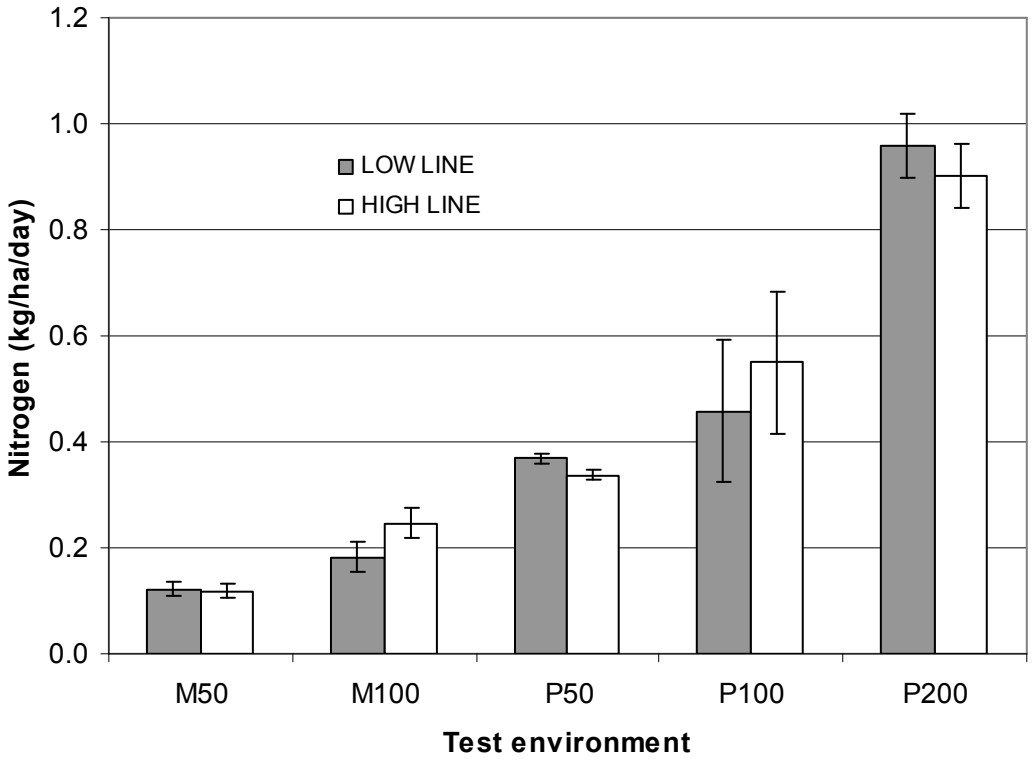

Figure 2. Average amount of nitrogen input in each test environment as calculated from the actual amount of protein pellets or chicken manure supplied to the ponds (minimum and maximum values of the replicate ponds indicated by the error bars).

The amount of nitrogen supplied in each test environment is shown in Figure 2. There was an increase in the amount of nitrogen input from test environment M50 to M100 to P50 to P100 and P200 but the amount of nitrogen applied differed between replicate ponds and between selection lines.

The Type III mean squares of the effects in Model 1 are shown in Table 2. All effects were highly significant ($P < 0.001$). Sex and initial body weight were the main sources of variation in body weight. Model 1 explained 34% of the variation in body weight at harvest. The estimated effects for line by test environment interaction of body weight at harvest are shown

in Figure 3. The high line performed better than the low line in all test environments except in P100 where there were no differences in performance. Although the lines showed a similar trend in performance, the low line showed less fluctuation over the test environments.

Table 2. Type III mean squares (MS), degrees of freedom (DF) and the significance of effects for body weight at harvest according to Model 1

Source	DF	MS	P- value
Line	1	5.46	P<0.001
Test environment	4	8.91	P<0.001
Sex	1	72.89	P<0.001
Pond(line*test environment)	20	1.55	P<0.001
Age	1	5.07	P<0.001
Log initial weight(line)	2	14.89	P<0.001
Line*Test environment	4	3.81	P<0.001
Error	3668	0.10	
R^2	0.34		

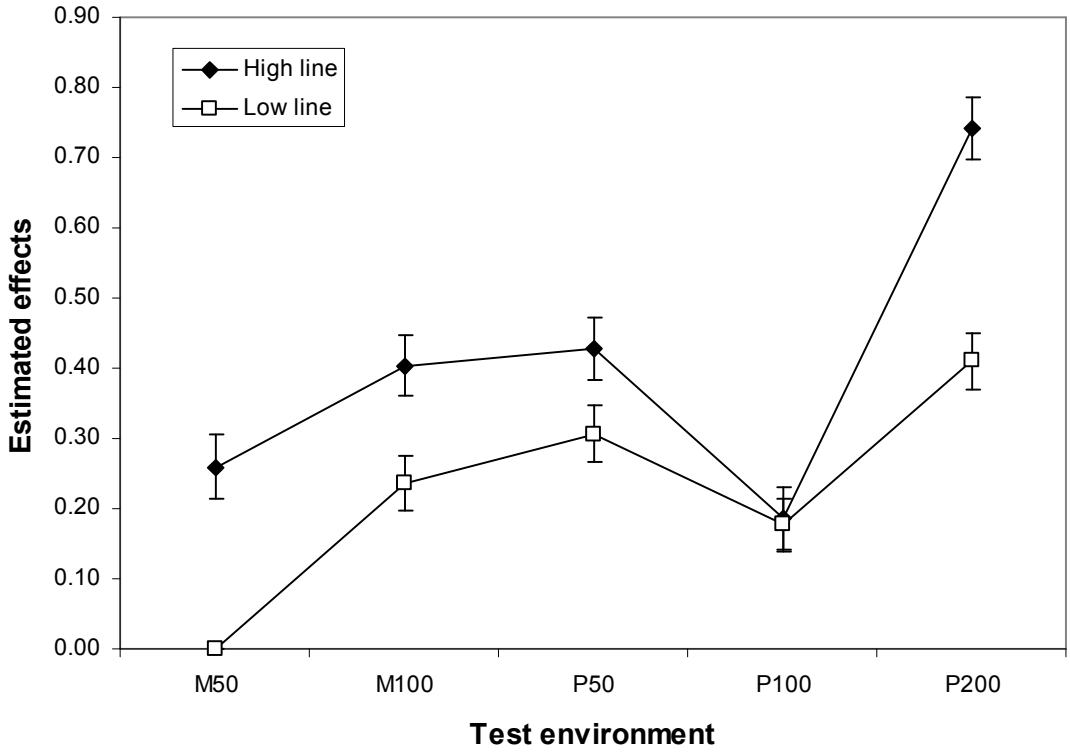

Figure 3. Estimated effects for line by test environment interaction of model 1 for the log-body weight (standard errors indicated by error bars)

Results of the analysis for both of the selection lines according to model 2A are shown in Table 3. Both lines showed a significant family by test environment interaction effect. Sex by test environment interaction was only significant in the high line. As shown in Figure 4, the sex by environment interaction did not involve re-ranking of sexes across test environments. In the high line, Model 2B without test environment or interaction terms had an R^2 of 0.41, when nitrogen was included the model (including the quadratic term) the model had an R^2 of 0.54, and with test environment in the model the R^2 became 0.60. By replacing test environment with nitrogen 6% of the variation attributed to test environment could not be explained while in the low line 5% of the variation due to test environment could not be explained. This indicates that nitrogen was a poor descriptor of the test environment. When test environment was replaced by dissolved oxygen, there was a 15% reduction in R^2 in the high line and an 11% reduction in R^2 in the low line. This shows that, although nitrogen was a better descriptor of the test environment than dissolved oxygen, neither of these factors could completely describe the effect of test environment. We only observed a significant family by nitrogen (P<0.001) and family by dissolved oxygen interaction (P= 0.028) in the low line.

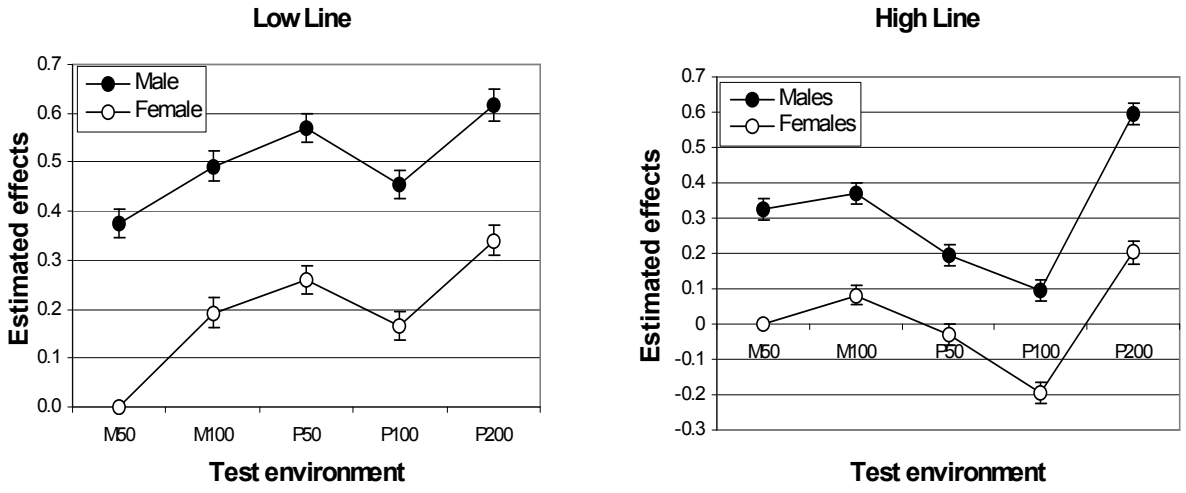

Figure 4. Estimates of sex by test environment effects of log-body weight at harvest for male and female Nile tilapia from low and high line according to Model 2A (Standard errors indicated by error bars).

In general, there were higher Pearson correlation coefficients between test environments in the high line than in the low line. The Pearson correlation coefficients between family means in different environments can be translated into expected genetic correlations (equation 1)

when assuming that the heritabilities in both environments are 0.4 and that the family means are based on 10 offspring. For example, we can expect the Pearson correlation coefficient of 0.67 between test environment M50 and M100 in the low line if the genetic correlation between growth performances in both environments is equal to 0.85. The lowest correlation between family means in test environment P200 and M100 (i.e. 0.44) corresponds to a genetic correlation of 0.56, which supports the observed family by test environment interaction (Table 3). In the high line, the lowest Pearson correlation coefficient (0.72, between M100 and P100) corresponds to a genetic correlation of 0.92. This also supports the low family by test environment interactions observed in the high line.

Table 3. Type III mean squares (MS), degrees of freedom (DF) for the effects in model 2A for log-body weight and the significance of effects in low and high-input lines

	Low line			High line		
Source	DF	MS	Pr > F	DF	MS	Pr > F
Family	56	0.68	<.001	38	1.86	<.001
Test Environment	4	2.97	<.001	4	8.52	<.001
Sex	1	37.18	<.001	1	36.21	<.001
Pond (Test environment)	10	1.65	<.001	10	1.43	<.001
Log initial weight	1	6.00	<.001	1	0.79	0.001
Age	1	0.48	0.008	.	.	.
Family*Test Environment	194	0.11	<.001	156	0.79	0.069
Sex*Test environment	4	0.06	0.506	4	0.23	0.006
Error	1666	0.068		1515	0.065	
R^2		0.58			0.65	

Table 4. Pearson correlation coefficients between estimates of log-body weight at harvest for the full sib family by test environment interaction in model 2A: Low line above diagonal; high line below diagonal

	M50	M100	P50	P100	P200
M50		0.67	0.59	0.56	0.44
M100	0.83		0.54	0.62	0.45
P50	0.83	0.90		0.55	0.55
P100	0.78	0.72	0.74		0.61
P200	0.89	0.82	0.86	0.83	

4. Discussion

4.1 Methods

The main objectives of the present study were to compare the growth of two selected strains of Nile tilapia across a range of environments and to test their sensitivity to environmental changes. To this end, a common genepool was first formed and divided in two groups which were then selected simultaneously for two generations in fertilized or pellet-fed ponds. Earlier studies in Nile tilapia were limited to testing wild and farmed strains prior to starting a selection program. For example, Eknath et al. (1993) and Bentsen et al. (1998) carried out similar experiments in which Nile tilapia were tested over several test environments. In the present study, we used test environments that differed by the type and amount of nitrogen input. The set-up in the present study enables us to determine the effect of the selection environment on the performance of the selected strains under potential farming conditions.

To detect GXE, environments can be classified into groups and a genotype by environment interaction term included in the traditional quantitative genetic model, or the expression of the trait in different environments may be defined as separate traits. A high genetic correlation indicates that the traits are controlled by the same set of genes (Falconer and Mackay, 1996) and that there is no GXE. When phenotypes change gradually and continuously over an environmental gradient, GXE may be described by a reaction norm model (de Jong, 1995). The reaction norm model describes the phenotype expressed by a genotype as a function of the environment. In the present study, we could not directly estimate the genetic correlation between test environments due to small sample size within test environments. However, as a first step towards modeling a reaction norm, we attempt to determine variables that describe the pond environment on a continuous scale with nitrogen and dissolved oxygen as potential descriptors. In this study, the test environment could not be fully described by nitrogen or dissolved oxygen indicating that other factors are also involved. These factors may relate to the palatability of the feed provided as well as the presence of some natural food in the pellet-fed ponds.

4.2 Early growth and environmental effects

In the present study, the initial body weight for the low line was much larger than that for the high line although their ages did not vary as widely. This may be surprising considering that

the high line received high protein feed. In an earlier study in hapa-in-pond environments (Charo-Karisa et al., in press), we showed that the rearing environment had a large effect on fry performance. The use of high protein pellets as is used for nursing the high line increases hapa fouling (Bhujel, 2000). The high line may have experienced water quality problems in the concrete tanks due to clogging and hapa fouling; something not experienced in the low line.

4.3 Genotype and sex by environment interactions

We found significant line by test environment interaction that did not involve rank changes of lines in different test environments. Several authors reported low but significant strain by test environment interaction in Nile tilapia (Eknath et al., 1993; Macaranas et al., 1997; Bentsen et al., 1998). As noted by these authors and also in the present study, most of the variation in Nile tilapia is a result of sex (dimorphism) and initial weight differences. In the present study the selection lines had been selected in different environments for only two generations. The observed line by test environment interaction is expected to be stronger after more generations of selection.

We found significant family by test environment interactions in the low line but not in the high line. The absence of genotype by environment interaction in the high line was also confirmed by the Pearson correlations (Table 4). The presence of family by test environment interaction in the present study agrees with our earlier study (Charo-Karisa et al., in press) in which we found indications for genotype by environment interaction for early growth of Nile tilapia juveniles whose parents had been selected for growth in fertilized ponds. In the present study we found significant interaction of family with nitrogen and dissolved oxygen, indicating that the family by test environment interaction relates to the responsiveness of the low line to dissolved oxygen and nitrogen. In an earlier study, we also observed that the genotype by environment interaction was related to the level of dissolved oxygen (Charo-Karisa et al., in press).

The high sex effect in the present study was in agreement with earlier studies in Nile tilapia (e.g. Eknath et al., 1993; Macaranas et al., 1997). We also found a sex by test environment interaction in the high line but not in the low line (Table 3). Significant sex by test

environment interaction has been reported in earlier studies in Nile tilapia (Eknath et al., 1993; Macaranas et al., 1997; Bentsen et al., 1998). However, judging from Figure 4, it is clear that in the present study the sex by environment interactions were not biologically relevant.

One of the objectives of the present study was to test for the sensitivity of the two lines across test environments. Falconer (1990) suggested that to increase the mean performance of a genotype over a range of environments, selection should be done upwards in the less favourable environment because this reduces the sensitivity of the genotypes. We found evidence of differences in sensitivity of the selection lines. Although within the high line we found no evidence of family by environment interaction, its performance fluctuated widely over the test environments (Figure 3). Further, Figure 3 suggests that, as a strain, the low line is more stable. It should however be noted that the performance of test environment P100 was lower than expected in the present study. The feed used for the rearing of the high input line during selection was similar to the one used in P100 test environment in the present study. Muendo et al. (2006) compared the growth of fish in manure fertilized ponds (M100) against the same feed (P100) and found better growth in M100 than in P100. This indicates that the test environment for the "high" selection line probably was less high than planned and that the two environments were in fact rather close. To test for Jinks-Connoly rule, the high input environment should be more favorable than the P100 used in this study.

One of the aims of the present study was to generate data that can be used to support the decision on the best environment for selection of Nile Tilapia for resource-poor farming conditions. Although results indicate that the high line performed better across test environments we noted that it fluctuated more than the low line across test environments. The decision on the best environment depends upon the genetic correlation and heritabilities of the trait in the two environments (e.g. Mathur, 2002). Our data did not allow for direct estimation of the heritability or the genetic correlation between test environments. Further analysis is needed to answer this question more satisfactorily. It is clear from this study however, that the selection environment can determine the performance of Nile tilapia strains. Given the high heritability for body weight in low-input ponds (Charo-Karisa et al., in prep) it is- from a genetic point of view- efficient to select in low input conditions.

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Chapter 8

General Discussion

1. Introduction

Farmed fish are produced in environments which vary widely depending on the culture site, the culture system and intensity, the levels of management applied, or in the combination of culture species used (Bardach et al., 1972). Selection for improved growth has typically been done under favorable conditions where fish were fed high protein pelleted feed. This might result in breeds which demand more resource than can be provided on the traditional fish farms, thus increasing cost of production (Moav et al., 1976). In resource poor regions, Nile tilapia is mainly reared under diverse small-scale systems in earthen ponds with daily organic fertilizers from livestock and occasional addition of bran or leftover food. To ensure the availability of consistently high quality genetic material for these wide ranging environments (Little, 2004), the design of a good breeding scheme and the choice of the best environmental conditions for selection is crucial. However, an important question is whether a single population or environment can be used to produce brood stock/fish seed for fast growth in different farming environments.

The aim of this thesis was to address this question and obtain parameters that can be used to form guidelines for a breeding program aimed at the production of improved broodstock and fish seed for resource poor fish farmers. From chapter II to chapter VII, the results of experiments investigating the prospects of selection for growth in Nile tilapia, and the actual implementation of a selection program in low input conditions are reported. The present chapter will, based on literature and the results described in previous chapters, discuss the prospects and requirements for establishing a practical breeding scheme for the small-scale farmer in resource poor regions of the world, with a focus on Africa. In doing this, a short review of the theory behind selecting for a wide range of low-input farming conditions will first be given, with a focus on phenotypic plasticity and the challenges associated with selecting for growth in low-input conditions.

2. Choice of selection environment

The theoretical framework for the present study was based on the Jinks-Connolly rule (Falconer, 1990) mentioned in Chapter I. This theory was tested by selecting two lines from a single population of Nile tilapia in high and low-input conditions for two generations. The high input environment consisted of ponds in which fish were fed with 25% fish protein in the form

of pellets. The low input environment consisted of ponds which were only fertilized with chicken manure. Before this study, no information on the breeding of fish, nursing of fry, or the expected response to growth in low-input conditions existed in literature. This study therefore began by determining whether it was possible to successfully select for growth in such low-input conditions.

As mentioned in Chapter I, selective breeding is traditionally done in high- rather than low-yielding environments because of slow growth rates in the low-yielding environment. This study illustrated, however, that fast growth can be achieved in low-input conditions. In Chapter II, it was shown that Nile tilapia juveniles can be grown at the same rate with or without supplementary feeds. Although the environment in which fish are reared has a great influence on growth, this was mainly caused by factors not directly related to type of inputs used (Chapter II). The heritability estimates for growth in the "good pond" in chapter II were very similar to those found in the main selection experiment (Chapter V) indicating that the expectations for low heritability in low-input conditions are unfounded. We found substantial response to selection after two generations of selection in the low input environment (Chapter V). Body weight was highly genetically correlated with body measurements (Chapter VI), indicating that when needed, alternative traits for measurement of growth can be used. Together, these results indicated that selection can be carried out effectively in low-input conditions.

In order to determine the comparative efficiency of selecting in low input environments, the low input line was compared with the high input line selected divergently from a common gene-pool. Results of Chapter VII clearly show that although the two lines were only selected for two generations in different environments, their performance differed across a range of environments. We found a significant line by test environment interaction and a significant family by test environment interaction in the low line, while sex by test environment interaction was found only in the high line. This indicates significant evidence for genotype by environment interaction in Nile tilapia. We found high heritability and response to growth rate under low-input conditions as well as a progressive increase in yields across generations (Chapter V), indicating that selection in low input conditions can be very successful. These results together suggest that- from a genetic point of view- there are no strong arguments

against selecting in a resource-poor environment. Furthermore, since the farmers use the same environment selecting in the same environment should be more efficient.

3. Phenotypic plasticity and correlated response

The ability of a single genotype to produce an array of phenotypes (or reaction norm) when exposed to a set of environments that differ in one or more aspects, is referred to as phenotypic plasticity (Schlichting, 1986; Stearns, 1989). The benefit of plasticity lies in the ability to produce a better phenotype-environment match across many environments (Levins, 1968). Although plasticity cannot be measured on a single individual, considerable evidence indicates that plasticity can be selected for (Scheiner, 2002). When an organism is faced with an environment which is less favourable in some aspects, trait values often change due to either adaptive plasticity or maladaptive stress response (Huey and Kingsolver 1989; Newman, 1992). These responses are expressed in alternative forms of morphology, behaviour or physiological states (West-Ebehard 1989). While it is generally agreed that natural selection should favour an organism that can produce an optimal phenotype in every environment it encounters, in reality organisms fail to respond optimally in every environment because of genotype by environment interactions and evolutionary constraints or trade offs (Gomulkiewicz and Kirkpatrick, 1992; Stearns, 1992).

One of the major problems that lead to reduction in growth rate in tilapia cultured in ponds is the tendency of females to mature and reproduce earlier and at small size. Consequently, it has been recommended that both faster growth rate and delayed age at maturation be included as the most important breeding goals for Nile tilapia (Bolivar et al., 1993). Allocating resources to growth or reproduction is a fundamental tradeoff in evolutionary life history and depends on environmental conditions (Stearns, 1992). Individuals in a population allocate their resources to somatic growth and reproduction depending on proximate environmental cues which are meant to confer fitness advantages in that particular environment.

The use of life-history theory as a tool to predict reaction norms for age and size at maturity was originally suggested and developed by Stearns and his co-workers (Stearns, 1983; Stearns and Crandall, 1984; Stearns and Koella, 1986) as a deterministic process. Later, Heino et al. (2002) introduced the concept of reaction norms for age and size at maturation as a

probabilistic process. These predictions sought to explain whether observed changes in age and size at maturity observed in organisms exposed to unavoidable growth stressors are adaptations that maximise fitness under given constraints. In sub-optimal or unpredictable environments, it is expected that natural selection will favor alleles directing a greater part of the energetic resources toward early reproduction (Stearns, 1992). Although there was a tendency for females to mature earlier in later generations in this study (Chapter VI), selection for growth performance did not affect early maturity, i.e. the two traits showed a low genetic correlation. In contrast to results of Chapter VI, Longalong et al. (1999) noted a high genetic relation between early maturity and fast growth of Nile tilapia in an experiment carried out with high protein fish meal.

Siems and Sikes (1998), working with fathead minnows (*Pimephales promelas*) found that resource allocation patterns change in response to food availability over short time scales. Larger Nile tilapia grown under fertilized pond conditions tended to have longer gut to length ratios (Chapter VI). Having longer guts may be a mechanism for the fish to be more efficient on a diet that is less nutritious (Bowen et al., 1995; Chapter VI this thesis), the consequence of increase in gut length on meat yield is not yet clear. For example, it could negatively affect the dressing percentage of the fish by increasing the amount of offal from Nile tilapia.

4. Establishment of breeding schemes for resource poor regions

The majority of global aquaculture production (approximately 90%) is currently in developing countries (Hishamunda and Ridler, 2002) with Africa contributing approximately 1% of the global production (Jamu and Brummet, 2004). Given the under-utilised water resources, available and inexpensive labour, high demand for fish, and suitable climate, there is a large potential for improvement of aquaculture in Africa. However, African aquaculture has had a long history of failure (Machena and Moehl, 2001). The failure for aquaculture to take off has been blamed on lack of clear policy and technological knowhow, inadequate infrastructure, poor extension support, lack of fish seed and feed, and poor financing (Hempel, 1993; Machena and Moehl, 2001).

About 95% of aquaculture production in Africa comes from small-scale farmers, with tilapia accounting for about 40% of total production. Small-scale production systems are

extensive/semi intensive utility oriented pond systems (100-500 m^2) operated by households and integrated with other agricultural activities (Machena and Moehl, 2001). In highly productive agricultural areas where land-subdivision has led to small parcels of land, small-scale aquaculture is the only option left. Targeting small-scale systems for improved production is therefore synonymous with increasing total production of the aquaculture sector in Africa. Currently, these systems are characterised by low yields and small fish size at marketing. Because tilapia prices in most cases depend on fish size and quality, farmed fish should be of acceptable standards (Adesulu, 2000; Afolabi et al., 2000). Hence, fish farming must compete with other agricultural activities for labour, water, and farm inputs at the farm level as well as with fish from capture fisheries at the market place.

Success in aquaculture depends on a viable concept, sound management, adequate financing and an organism suitable for the production system (Shultz, 1986). A major set-back to aquaculture production in Africa has been the problem of dependence on donor funding, making aquaculture unsustainable (Machena and Moehl, 2001). Kosgey et al. (2006) reviewed several small ruminant breeding programs among resource poor farmers in the tropics and highlighted issues determining their success or failure. One important issue causing failure is that of top-down approaches in setting breeding goals and insufficient involvement of farmers which leads to setting up of wrong breeding goals i.e. not suitable for the target conditions, and lack of continual monitoring of animals at the multiplier level. These issues may apply in selective breeding programs for fish breeding as well. Management practices and breeding goals in resource poor regions may differ from those in favourable environments. The choice of appropriate selection strategy should therefore be informed by farmer needs, the production environments, and climatic conditions.

4.1 Choice of selection strategy

Several selection experiments and breeding programs aiming at increased growth rates have recently been conducted in *O. niloticus* under favourable environments (Hulata et al., 1986; Brzeski and Doyle, 1995; Eknath et al., 1993; Bentsen et al., 1998; Bolivar and Newkirk, 2002). The initial low response reported for tilapia produced through mass selection programs was attributed to the lack of effectiveness of mass selection and the use of genetically poor strains (Tave and Smitherman, 1980, Hulata et al, 1986, Behrends et al., 1988; Teichert-

Coddington and Smithermann, 1988; Huang and Liao, 1990). Therefore one of the most successful tilapia breeding programs, the Genetically Improved Farmed Tilapia, (GIFT) used combined family and within family selective breeding strategy and wild Nile tilapia stocks from Africa (Dey and Gupta, 2000). Nile tilapia originates from Africa and therefore there are good prospects for starting breeding programs with a good genetic base. Currently, there are no real domesticated strains of Nile tilapia in Africa. It has been suggested that the most efficient method for selection in Nile tilapia is family selection or BLUP selection (Uraiwan and Doyle, 1986; Gall and Bakar, 2002). However, recent reports indicate that mass selection can be used with good response in Nile tilapia (Basiao and Doyle, 1999; Basiao et al., 2005). Mass selection is a simple and cheap strategy to realise improvement when emphasis is on a single trait. A major advantage of BLUP over mass selection is that it allows for collection and use of information on relatives and other traits. It can be useful when not all traits can be measured on the selection candidate, for example for carcass traits and for performance in different environments. The choice of selection method should therefore depend on the economic capabilities of the community as much as on the selection criteria.

Rutten (2005) discussed the advantages and disadvantages of various selection strategies in terms of the necessary facilities, workload, inbreeding levels, and selection response. He found that under recirculation systems, both BLUP selection and mass selection required basically the same facilities, and labour, the only difference being the extra cost of tags in BLUP. In the present study, we used fewer facilities than would normally be required under standard hatchery conditions. In standard hatcheries using hapa-in-pond systems, e.g. the GIFT project each full-sib family requires two or more hapas, one at the spawning phase and the others at the rearing phase; the rearing phase requiring hapas with larger mesh size. In the present study, a single hapa per full-sib family was used at both the spawning and the rearing phases before tagging. To reduce the workload, each full-sib family was tagged as soon as fry reached 2 g mean weight (Chapter V). This way, the tagging work was spread over the rearing period and only a few workers and minutes were required at any given time. Because no feed was added, hapa fouling was greatly minimized which reduced the frequency of hapa cleaning. A total of 100 hapa and two ponds were required to produce and rear 100 full sib families. Each generation, a little over 2000 fish were reared in two ponds. Before selection of brooders, the harvested fish were measured and returned into the same hapa in ponds, requiring no further

holding facilities. The minimal number of facilities used and the sub-optimal amounts of manure applied in this study makes this approach appropriate for resource poor regions.

4.2 Founder stocks

Genetic variability is crucial in the breeding program because this is critical for both the short-term and long term limits of response (Falconer, 1989). A synthetic founder population incorporating wild strains of wide genetic variability and suitable production traits could be formed first. This involves comparing the growth performance and survival of pure strains in potential farm environments with the aim of producing domesticated strains from best performing wild strains only. Crucially important also is the development of strategies to maintain the variability (i.e. to limit the rate of inbreeding) through generations of selection. This means that a relatively large breeding population needs to be used in which selected parents are not highly related.

4.3. Environment of selection

Whereas the highly intensive culture practiced in developed countries relies heavily on high protein pelleted supplemental feeds, protein sources are limited in most developing countries. In fertilized ponds, as used in the present study, the diet consists of mainly phytoplankton. This diet has limited protein, tends to reduce assimilation efficiency, and is generally less favorable to fish growth (Bowen et al., 1995). Manure fertilization of ponds also promotes development of benthos such as chironomids and oligochaetes (Friday, 1987; Kullberg and Peterson, 1987) which apart from playing a significant role in the release of nutrients are directly eaten by tilapia (Egna et al, 1997; Teichert-Coddington et al., 1997). By providing substrates in the fertilized ponds, the growth of periphyton can be increased and this significantly improves fish yields (van Dam et al., 2002) which indicates more scope for improving the productivity of low-input fertilized ponds. A two pronged approach to improving tilapia yields with only small extra cost i.e. improving natural pond productivity and the genetics of the fish can be applied successfully in low-input ponds.

Feed takes up more than 50% of the operation cost of intensive tilapia culture (El-Sayed, 1998; Wu et al., 1999). For nursing of fry 40% protein pellets are used. Apart from being expensive (during the study 40% protein pellets cost 3.0 Egyptian pounds (L.E.)), these high protein

pellets are not readily available in resource poor regions. Therefore, from an economic point of view a hatchery that uses manure instead of pellets would spend less and thus produce cheaper seed and broodstock. Similarly, a farmer that uses manure at the grow-out period spends less than the one using pellets. To illustrate this we compare the input amounts used for a 200 m^2 pond (Chapter VII) for test environment M100 (50kg/ha/day chicken manure) and P100 (25% protein pellets). On average, each of the M100 ponds received 1 kg chicken manure daily while the P100 ponds received 0.25 kg pellets per day. Chicken manure in Egypt is sold at 250 L.E. per ton or 0.25 L.E. per kg, and 25% protein pellets is sold at 2 L.E. per kg. For a grow-out period of 180 days, the farmer/breeder using chicken manure spends 45 LE per pond while the farmer/breeder using pellets spends twice as much (90 LE). Integration of fish farming with poultry or other livestock increases opportunities for saving and is thus more cost-effective.

4.4. Delivering quality seed to farmers: a participatory approach to farmer involvement

A major setback to farming of tilapia in resource poor regions has been the lack of quality fish seed (Machena and Moehl, 2001; Little et al., 2001; Little, 2004). In most resource poor regions, delivery of fry at the farm gate as well as the unavailability of the high quality fry from research stations are important challenges (Little, 2004). Production and dissemination of fish seed can be either centralised or decentralised. In the centralised approach, the breeding program is located in a central place and farmers get the fry through multiplier centres. The advantage of this approach is that genetic quality can be easily maintained. The disadvantage is that the rural communities remain dependent on a distant source which may become unrealiable (Little, 2004). Inconsistent performance of fish may arise due to hatcheries that provide seed of variable age and size from partial, asynchronous spawning of broodfish (Little, 1998; Little and Hulata, 2000). If the centralised system must be used, multiplier centres that are manned by the local farmers may be an appropriate alternative. In the decentralised approach, the juveniles are produced close to the grow-out areas allowing farmers to have a greater control of the seed availability. Because of the problem with infrastructure in most of Africa, the decentralised approach seems a better option. This has the advantage of cutting on transport cost and has been used successful in Asia (Little, 2004). Splitting the breeding program in two or more separate populations will allow for exchange of broodstock at a later stage which supports the maintenance of variability within populations. These populations could also act as a backup in case of accidental loss through disease outbreaks or natural disasters (Bentsen and Gjerde,

1994). However, it should be noted that in case the breeding program is too dispersed, the need to tag animals and evaluate breeding stocks at many different locations could increase costs.

In establishing a sustainable breeding program it is recommended to involve local fish farmers from inception of the breeding program onwards. Involving farmers from the program inception could help to tackle the perception that aquaculture is technically complex and difficult to handle (Machena and Moehl, 2001). A participatory approach to farmer involvement will enable farmers to conceive the breeding program as their own, and if aquaculture extension officers are involved can be used simultaneously to train farmers on general aquaculture practices. By allowing farmers to define the breeding goals at the onset, the breeding program ensures that the resultant breeds match local conditions and needs, thus ensuring program sustainability. Ambali and Malekano (2004) pointed out that selective breeding programs are likely to be abandoned in Africa because they are expensive, and because it takes long to improve a strain through selective breeding. However, there was a progressive increase in size of the fry produced across generations in this study (Chapter V). We further demonstrated that a breeding program does not necessarily have to be expensive. What is required is the commitment of farmers to participate. Nile tilapia farmers, who are accustomed to using unimproved seed, are likely to be encouraged as they use improved seed after every generation of selection.

In aquaculture, some traits of major economic importance include growth rate and meat yield, feed conversion efficiency, disease resistance and survival, flesh quality, and age at sexual maturation (Gjedrem, 2000). In small-scale farms, the most important breeding goal is improved growth. In fertilized ponds, survival is one of the most important traits for improvement and is substantially heritable (Chapter V). Schultz (1986) recommends minimizing the number of traits to be improved to minimize costs of trait measurement and to keep the breeding program focused. This may be more relevant in resource poor regions. Therefore body weight and survival should be the first target traits for improvement. Because of the high genetic correlation between body weight and body measurements (Chapter VI), body measurements, which are easier and more convenient to measure, may be used as the traits of selection. In sub-tropical and high land areas in Africa, growth is often hampered by low temperature. Because of low prospects of improvement of this trait through selective

breeding (Chapter III), it was recommended that focus should be on increasing body weight of fry before stocking and the acclimatization of fry to cold temperature before grow-out. Further study is needed to determine whether acclimatization effects are maintained till harvesting. Because farmers are involved in the breeding programs, emerging traits of concern may be identified and the breeding scheme adjusted accordingly.

5. Conclusion

This thesis has demonstrated that there are good prospects for setting up sustainable breeding programs for resource poor tilapia farming conditions without requiring expensive supplementary protein pellets. Because poverty alleviation and food security are primary goals in the developing world, the initiation and implementation of cheaper breeding programs will ensure that the genetically improved material are accessible to the rural fish farmer. High response can be achieved within a few generations in Nile tilapia, and because of short generation times, fish farmers can reap the benefits of genetic improvement programs without delay thus reducing the risk of failure of the breeding program. Appropriate breeding goals for these breeding schemes need to be set-up with the involvement of the local farmers, preferably for each agro-ecological zone (but within the financial capabilities of the community), to ensure that the breed meets the requirement of local farmers. The formation of multiplication centres where farmers can collect fry or a decentralised breeding program could ensure that the improved fish seed gets to the farm gates when required. Full benefit of the breeding program will be realised by improving husbandry practices that enhance water quality and improves nutrition such as the use of substrates that provides surface area for growth of periphtyon. It is important that researchers and aquaculture extension officers are trained on how to keep records because although record-keeping is a tool for monitoring progress it has not been a common practice for fish farmers to keeps proper records. This is a necessary step for the maintenance of domesticated strains. The initiation and implementation of a breeding program as described in this thesis can still be expensive for the rural poor; however donor dependence should be discouraged. Instead, the role of governments in forming policies that make it easier for fish farmers to access credit should be enhanced. This way, fish farmers can also learn how to manage their own finances or management of corporate breeding schemes.

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Summary (English, Dutch, Kiswahili)

SUMMARY

Among the tilapias, Nile tilapia is the most important species farmed in the world and is the mainstay of many resource-poor fish farmers. The majority of its culturing is carried out in the tropics in semi-intensive environments with a wide array of pond inputs from the farm. To increase production, the overall efficiency of fish reared in these systems needs to be improved. Studies have shown that whereas about 15-30% of the nutrient input in pellet fed-pond systems is converted into harvestable products, only 5-15% of the nutrient input in fertilizer-only pond systems is converted to harvestable products. This thesis was a part of the INREF-POND project (Chapter I), which consisted of sub-projects at the fish, pond and farm level with experimental and modelling studies. The aim of the project was to find ways of increasing the efficiency of nutrient use of integrated systems. Efficient breeding programs are needed to improve the overall nutrient use efficiency of fish in fertilized ponds. This thesis aims at (i) studying the feasibility of selective breeding for the improvement of growth of Nile tilapia in low-input extensive farming conditions, (ii) determining the effects of selection on other performance traits and (iii) investigate genotype bye environment interaction in Nile tilapia.

A common practice in tilapia farming is to nurse fry in hapa-in-pond systems with high protein pellets. To carry out a selective breeding in low-input condition, knowledge of factors determining growth in these systems is important. **Chapter II** presents results of an experiment that was carried out to determine the optimal conditions for early rearing of tilapia fry in hapa-in-pond systems. The aim of this study was to quantify the environmental and genetic effects on early growth of Nile tilapia, in hapa-in-earthen pond systems. In a pilot study, we grew swim-up fry with or without supplementary feed in hapas suspended in fertilised ponds at 5, 10, 15, and 20 fry/m^2 densities. In the main experiment, we reared swim-up fry from 25 full-sib families separately for 42 days at 15 fry/m^2 density in hapas suspended in two earthen ponds. Hapas were arranged in two column arrays along the sides of the ponds. Ponds were fertilized daily with chicken manure. In addition, fry in one column in each pond were fed twice daily on 40% protein pelleted feed. Results from the pilot study indicated significant effects of stocking density and treatment on fry growth. In the main experiment, the dietary treatment effect was not significant but there were large differences in growth between ponds: mean body weight at 42 days was 1.7 g in pond A and 0.4 g in pond B. Heritability (h^2) of 42-day fry body weight estimated from the whole data set using a

univariate model was 0.01±0.06. The bivariate heritability estimates were 0.59±0.19 in pond A and 0.05±0.11 in pond B. The common environmental / hapa (c^2) effects were 0.14±0.06 and 0.29±0.07 in respective ponds. We found significant positive spatial autocorrelation (P = 0.02) indicating resemblance in growth of fry in neighboring hapas. Analysis of environmental variables showed that the two ponds differed significantly in dissolved oxygen. The low genetic correlation (r_g = -0.27±0.69) between body weights of fry in both ponds therefore might suggest genotype by environment interactions for tolerance to low dissolved oxygen in Nile tilapia juveniles.

The inability of tilapia to tolerate low temperatures is of major economic concern as it reduces their growing season and leads to over winter mortality. In **Chapter III**, cold tolerance of juvenile Nile tilapia was investigated and heritability estimates obtained. Eighty full-sib families were produced by mating each sire with two dams. Fry were grown in hapas suspended in earthen ponds fertilized with chicken manure, and were 41-91 days post-hatch at the start of the experiment (mean standard length 50.6 mm; mean body weight 5.1 g). Fry were tagged and exposed to low temperature in an indoor facility. Temperature was lowered from 16 °C to 11°C in 48 hours and from 11 °C to 8 °C at the rate of 1°C/day. Cold tolerance was expressed as Temperature at Death (TAD) and Cooling Degree hours (CDH). Fish mortality started at 13.6 ºC and total mortality occurred at 8.6 ºC. Mean TAD and CDH were 10.1 ºC and 298.07 respectively. Fish body weight (BW) had a highly significant effect on cold tolerance (P <0.0001). Smaller fish (<5g) were more susceptible to lower temperature than larger fish. The heritability of cold tolerance was 0.08±0.17 for CDH and 0.09±0.19 for TAD, estimated with an animal model. There was a considerable common environmental/full-sib effect for this trait (0.33±0.10 for CDH and 0.27±0.09 for TAD). These values indicate that estimation of genetic parameters for cold tolerance in tilapia should include both direct additive and common environmental effects. Based on the results of this study we conclude that the most appropriate way of enhancing cold tolerance of tilapia juveniles is by husbandry practices that increase pre-winter body weights.

The effects of genotype, age, size, condition factor and diet (natural phytoplankton versus formulated protein pellets) on low-temperature tolerance of juveniles were investigated and reported in **Chapter IV**. This chapter compared the results of the experiments reported in

Chapter III with a second experiment carried out to determine the effect of the environment and diet on cold tolerance. In the first experiment, 775 juveniles from 43 sires and 80 dams were reared under mid-summer conditions for 41-91 days. In the second experiment, 393 juveniles were produced by single-pair mating of 20 dams and 20 sires from the same brooders as in the first experiment. These fish were reared for 42 days under autumn conditions with either high protein (40%) pellets or natural tilapia diet. At the end of the growth period fish from each experiment were tagged and exposed to gradually lowered temperatures. Cold tolerance was significantly affected by genotype, size, aquarium, condition factor (P= 0.0001) and diet (P=0.0547). In both experiments, smaller fish were more vulnerable to cold stress. Age did not significantly affect cold tolerance. Fish reared under mid-summer conditions died between 13.6 ºC and 8.6 ºC while those reared under autumn conditions died between 11.7 ºC and 7.5 ºC. This suggests that acclimatization to lower temperatures before cold stress can improve the cold tolerance ability of *O. niloticus*.

Knowledge of the heritability for growth and the correlated changes that occur in other traits due to selection for growth is important for the design of an efficient genetic improvement program. In **Chapter V** and **Chapter VI** estimates of phenotypic and genetic parameters for growth, body measurements, reproductive traits and gut length from two generations of selection for growth in a low input environment are presented. The selection environment consisted of earthen ponds which were daily fertilized with 50 kg dry matter (dm) /ha chicken manure. No supplementary feeds were provided. In total, 6429 fully pedigreed experimental fish were included in the analysis. Survival till harvest was highly variable ranging from 35% to 77% and was affected by initial weight, pond, and age effects. Body weight at harvest (BW) increased from a mean of 67.4 g in the grandparental (unselected) population (G_0) to 129.5 g in G_2 was affected by initial weight, pond, sex and age effects. Generations were discrete and therefore genetic parameters were estimated separately for each year. Heritability estimates for BW ranged from 0.38 to 0.60, and the heritability for survival ranged from 0.03 to 0.14. The estimated selection response was 23.4 g (34.7%) between G_0 and G_1 and 13.0 g (14.9%) between G_1 and G_2 (Chapter V). **Chapter VI** presents the estimates of phenotypic and genetic parameters for body size measurements, reproductive traits, and gut length. Heritability estimates for body measurements ranged from 0.4-0.6 for standard length to 0.69-0.79 for head length. Phenotypic correlations between body weight and body

measurements ranged from 0.64 to 0.89. Genetic correlations were close to unity. The heritability estimate for maturity at harvest was 0.13. Heritabilities for carcass traits were estimated from G_1 only and were 0.16 for gutted weight and 0.06 for dressing percentage. Phenotypic correlation between body weight and gutted weight was 0.84 and the genetic correlation was 0.20. Gut length increased with selection for body weight. Heritability estimate for gut length was 0.22. Moreover, gut length and body weight were genetically highly correlated. These results demonstrate the feasibility of selection for growth of Nile tilapia in low-input environments.

The effect of selection environment on the performance of selected strains over a range of potential production environments is a fundamental breeding question. In the presence of genotype by environment interaction, genetic improvement obtained by selection in one environment may not be realized in other environments. In **Chapter VII,** the results of a study designed to determine the extent of genotype by environment interaction in Nile tilapia are presented. We compared the performance of the low line with a high line selected for growth in ponds where fish received 25% protein. The 2 lines were tested in five test environments: 40% protein pellets feed (P200), 25% protein pellets (P100), 16% protein pellets (P50), 50 kg/ha chicken manure (M100) and 25 kg/ha chicken manure (M50). Nitrogen input was similar in P50 and M50, and in P100 and M100 treatments respectively. Survival from stocking to harvest ranged from 70-75% in the high line and from 62 to 76% in the low line. Analyses revealed significant differences in growth performances of the two lines across test environments. The phenotypic mean body weight at harvest was highest for test environment P200 (123.4 g in the low line, and 131.7 g in the high line). Lowest phenotypic body weight means were 92.1g (test environment M50) in the low line and 82.4g (test environment P100) in the high line. Although the high line performed better in more test environments, there was a significant line by test environment interaction indicating that both lines were sensitive to the environment. Family by test environment interaction was significant only in the low line. This genotype by environment interaction was related to the interaction of the families to nitrogen and dissolved oxygen in the ponds.

These results described in this thesis show that there are good prospects for setting up sustainable breeding programs for resource poor tilapia farming conditions without requiring

expensive supplementary protein pellets. The prospects and requirements for establishing such a practical breeding scheme for the small-scale farmer in resource poor regions of the world are discussed in **Chapter VIII**. Because poverty alleviation and food security are primary goals in the developing world, it is important that cheaper breeding programs are initiated and implemented and the genetically improved material made accessible to the rural fish farmer. High genetic variability is crucial in the breeding program because it is critical for both the short-term and long term limits of response. Therefore, strategies to maintain the variability through generations of selection should be part of the breeding program. Appropriate breeding goals for these breeding schemes need to be set-up with the involvement of the local farmers, preferably for each agro-ecological zone to ensure that the breed meets the requirement of local farmers. The formation of multiplication centres where farmers can collect fry or a decentralised breeding program should ensure that the improved fish seed gets to the farm gates when required. Full benefit of the breeding program will be realised by promoting husbandry practices that use locally available crop residues and manure to boost the productivity of the pond and provide ample nutrients for the genetically improved breeds of Nile tilapia.

SAMENVATTING

Van alle tilapia soorten is de Nijl tilapia wereldwijd de meest gekweekte; voor arme boeren is het bovendien een belangrijke dierlijke eiwitbron. De vis wordt vooral gekweekt in de tropen, in semi-intensieve milieus zoals aarden vijvers die bemest worden met allerhande restproducten uit de landbouw en veeteelt. Om de produktie te verhogen dient de voederbenutting van de vis gekweekt in deze systemen verbeterd te worden. Studies hebben aangetoond dat 15-30% van de nutrienten, aanwezig in pellets, omgezet worden in een oogstbaar produkt. Bij vijvers die uitsluitend bemest worden is dit slechts 5-15%. Het onderzoek beschreven in dit proefschrift maakte deel uit van het INREF-POND project (zie hoofdstuk 1), wat uit drie deelprojecten bestond. Deze projecten bestudeerden het vis, vijver en bedrijfsniveau en waren onderling verbonden door modellerings studies. Het uiteindelijke doel van het INREF project was te onderzoeken hoe de efficiëntie van nutriëntgebruik op kleine geïntegreerde bedrijven verbeterd kon worden door gebruik te maken van visvijvers. Efficiente fokprogrammas zijn nodig om de voederbenutting van vis in bemeste visvijvers te verbeteren. Het doel van dit onderzoek was: 1) de haalbaarheid te onderzoeken van het fokken op groei van Nijl tilapia in laag bemeste vijvers; 2) te onderzoeken wat de effecten van een dergelijke selectie op groei voor andere kenmerken waren, en 3) te onderzoeken of er sprake was van genotype x milieu interactie.

Gewoonlijk worden tilapia larven in hapas, opgehangen in vijvers, opgekweekt en met eiwitrijke pellets gevoerd. Indien een selectieprogramma onder laag-input condities uitgevoerd moet worden is het belangrijk te weten welke faktoren larvale groei in dergelijke milieus bepalen. In hoofdstuk 2 worden resultaten beschreven van een experiment dat was opgezet om de optimale condities voor larvale opkweek in hapa-in- vijvers te bepalen. Het doel van deze studie was het kwantificeren van de omgevings- en genetische faktoren die de vroege groei van Nijl tilapia in hapas, opgehangen in bemeste aarden vijvers, bepalen. In een voorstudie werden pas uitgekomen larven met of zonder extra voer, in hapas, opgehangen in bemeste vijvers, opgekweekt bij dichtheden van 5, 10, 15 en 20 larven per m^2. In het hoofdexperiment werden pas uitgekomen larven van 25 full-sib families per familie gescheiden opgekweekt gedurende 42 dagen bij een dichtheid van 15 larven per m^2. In dit experiment waren de hapas in twee rijen aan weerszijden van de vijver opgehangen. In de ene rij werd bijgevoerd met 40% eiwit pellets, in de andere rij werd niet bijgevoerd. Het hele

experiment werd in duplo (2 vijvers) uitgevoerd. De resultaten van het voorexperiment lieten zien dat er significante effecten waren van behandeling (wel of niet voeren) en dichtheid op groei. In het hoofdexperiment was het effect van voeren niet significant, maar er waren grote verschillen in groei tussen de twee vijvers: het gemiddelde gewicht in vijver A was 1,7 gram terwijl dat in vijver B slechts 0,4 gram was. De erfelijkheid van lichaamsgewicht op een leeftijd van 42 dagen, geschat op de hele dataset en met een univariaat model, was 0.01 ± 0.06. De bivariate schattingen waren 0.59 ± 0.19 in vijver A en 0.05 ± 0.11 in vijver B. Het effect van gemeenschappelijk milieu (c^2 = hapa plus familie effect) was achtereenvolgens 0.14 ± 0.06 en 0.29 ± 0.07 in beide vijvers. We vonden een significant positieve autocorrelatie tussen nabij gelegen hapas, wat er op duidt dat de groei in twee aan elkaar grenzende hapas op elkaar lijkt. Een analyse van milieu faktoren toonde aan dat de twee vijvers vooral in de hoeveelheid beschikbare zuurstof van elkaar verschilden. De lage genetische correlatie (r_g = -0.27 ± 0.69) tussen lichaamsgewicht van larven in beide vijvers suggereert genotype x milieu interactie voor lage zuurstof tolerantie in Nijltilapia larven.

Nijl tilapia zijn weinig koude resistent. Dit is een economisch belangrijk probleem voor subtropische gebieden waar koude perioden het groeiseizoen verkorten en winter mortaliteit veroorzaken. In hoofdstuk III, werd de koude tolerantie van juvenile Nijl tilapia onderzocht en werd de erfelijkheid van koude tolerantie geschat. Tachtig full sib families werden geproduceerd door steeds 1 mannetje met 2 vrouwtjes te paren. Larven werden opgekweekt in hapas in aarden vijvers, bemest met kippenmest, en waren 41-91 dagen oud bij het begin van het experiment (gem.lengte 50.6 mm; gem. lichaamsgewicht 5.1 gram). De jonge visjes werden gemerkt en blootgesteld aan lage temperaturen in een koelcel. De temperatuur werd gedurende 48 uur verlaagd van 16 ºC tot 11 ºC, en daarna van 11ºC naar 8ºC met stappen van 1 ºC /dag. Koude tolerantie werd uitgedrukt als “temperature at death (TAD)” en “cooling degree hours (CDH)”. Sterfte begon op 13.6 ºC en was bij 8.6 ºC volledig. Gemiddelde TAD en CDH waren respectievelijk 10.1 ºC en 298.07. Lichaamsgewicht had een significant effect op koude tolerantie. Kleinere vissen (<5 gram) waren meer gevoelig voor lage temperaturen dan grotere vissen. De erfelijkheid van koudetolerantie, geschat met een animal model, was 0.08 ± 0.17 voor CDH en 0.09 ± 0.19 voor TAD. Er was een aanzienlijk effect van gemeenschappelijk milieu + familie voor dit kenmerk (0.33 ±0.10 voor CDH en 0.27 ± 0.09 voor TAD). Deze waarden tonen aan dat de schatting van genetische parameters voor koude

tolerantie zowel additief genetische als gemeenschappelijke effecten moet betreffen. De beste manier om koude tolerantie in Nijl tilapia te verbeteren is door, via verbeterde houderij, te streven naar hogere lichaamsgewichten voorafgaand aan de winterperiode.

In hoofdstuk IV worden de effecten van genotype, leeftijd, gewicht, konditie factor en dieet (natuurlijk fytoplankton versus eiwitrijk voer) op de koudetolerantie beschreven. In dit hoofdstuk worden de resultaten van het experiment beschreven in hoofdstuk III vergeleken met de resultaten van een tweede experiment waarin specifiek naar het effect van omgeving en dieet gekeken werd. In het eerste experiment werden 775 juvenielen, afkomstig van 43 vaders en 80 moeders, opgekweekt bij zomer temperaturen gedurende 41-91 dagen. In het tweede experiment werden 393 vissen geproduceerd door een op een kruisen van 20 vrouwtjes met 20 mannetjes. Deze vissen werden gedurende 42 dagen onder na-zomer/herfst temperaturen opgekweekt. Gedurende deze periode werd een deel van de vissen gevoerd met een 40% eiwit pellet, terwijl een tweede groep alleen natuurlijk vijver voedsel tot zijn beschikking had. Aan het eind van de groeiperiode werden de dieren gemerkt en overgebracht naar een aquarium in een koelcel waarin de temperatuur geleidelijk omlaag gebracht werd. Koude tolerantie werd significant beïnvloed door genotype, gewicht, aquarium, conditiefaktor ($P < 0.0001$) en dieet ($P = 0.0547$). Ook in dit experiment bleken kleinere vissen gevoeliger voor koude dan grote. Leeftijd hadden geen invloed op de koudetolerantie maar er was wel een effect van seizoen. Vissen,opgekweekt onder zomerse temperaturen stierven bij temperaturen tussen 13.6ºC en 8.6ºC, terwijl vissen opgekweekt bij herfst temperaturen stierven bij 11.7 – 7.5 ºC. Dit suggereert dat acclimatie aan lagere temperaturen voorafgaand aan de winter de koudetolerantie van Nijltilapia aanmerkelijk kan verbeteren.

Kennis van de erfelijkheid van groei en de gecorreleerde veranderingen die plaatsvinden in andere kenmerken als gevolg van selectie op groei is belangrijk voor het ontwerp van een efficiënt fokprogramma. In hoofdstuk V en hoofdstuk VI worden schattingen van fenotypische en genetische parameters voor groei, lichaamsmaten, voortplantings kenmerken en darmlengte gepresenteerd. Het selectie milieu bestond uit aarden vijvers die dagelijks bemest werden met 50 kilogram (droge stof) / hectare kippenmest. Er werd niet bijgevoerd. In totaal werden 6429 vissen met bekende afstamming gebruikt in de analyse. Overleving tot

oogst varieerde van 35% tot 77%. Overleving werd beïnvloed door begingewicht, vijver, en leeftijds effecten. Lichaamsgewicht bij oogst nam toe van gemiddeld 67.4 gram in de ongeselecteerde grootouder generatie (G_0) tot 129.5 gram in de tweede generatie van selectie, en werd beïnvloed door begingewicht, vijver, sexe en leeftijds effecten. Aangezien de generaties niet overlappend waren, werden de genetische parameters voor elke generatie afzonderlijk geschat. Erfelijkheid van lichaamsgewicht varieerde van 0.38 tot 0.6; de erfelijkheid van overleving varieerde van 0.03 tot 0.14. De geschatte selectie respons was 23.4 gram (34.7%) tussen G_0 en G_1 en 13.0 gram (14.9%) tussen G_1 enG_2 (hoofdstuk V). In hoofdstuk VI worden schattingen gegeven van fenotypische en genetische parameters voor lichaamsmaten, voortplantingskenmerken en darmlengte van Nijl tilapia. Erfelijkheidsgraden voor lichaamsmaten varieerden van 0.4-0.6 voor standaardlengte, tot 0.69-0.79 voor koplengte. Phenotypische correlaties tussen lichaamsgewicht en lichaamsmaten varieerden van 0.64 tot 0.89. Genetische correlaties waren allen bijna 1. De erfelijkheids schatting voor geslachtsrijpheid bij oogst was 0.13. Erfelijkheidsgraden voor slacht kenmerken werden alleen aan dieren uit generatie 1 geschat, en waren 0.16 voor uitslachtgewicht en 0.06 voor filetpercentage. De fenotypische correlatie tussen lichaamsgewicht en uitslachtgewicht was 0.84; de genetische correlatie was 0.20. Darmlengte nam toe met selectie op lichaamsgewicht. De erfelijksgraad schatting voor darmlengte was 0.22. Darmlengte en lichaamsgewicht bleken bovendien genetisch sterk gecorreleerd. Deze resultaten laten zien dat selectie op groei in bemeste vijvers zonder bijvoeren zeer goede perspectieven biedt.

De selectieomgeving is van groot belang bij het voorspellen van de prestatie van geselecteerde rassen over een groot scala aan potentiele productieomstandigheden. In het geval van genotype x milieu interactie kan de genetische verbetering, verkregen in een selectieomgeving, niet volledig gerealiseerd worden in andere milieus. In hoofdstuk VII worden de resultaten van een studie naar het belang van genotype x milieu interactie in geselecteerde Nijl tilapia gepresenteerd. We vergeleken de prestatie van de geselecteerde “lage lijn” (zie hoofdstuk V en VI) met die van een lijn, geselecteerd op groei in vijvers die bijgevoerd werden met 25% eiwit pellets (“hoge lijn”). Deze twee lijnen werden getest in 5 behandelingen: bijvoeren met resp. 40% eiwit pellets (P200), 25% eiwit pellets (P100) en 16% eiwit pellets (P50), of geen bijvoeren en alleen bemesten met resp. 50 kilogram ds / ha kippenmest (M100) of 25 kilogram ds / ha kippenmest (M50). De hoeveelheid stikstof was

hetzelfde in de P50 en M50 behandelingen, en in de P100 en M100 behandelingen. Overleving tot oogst varieerde van 70-75% in de hoge lijn tot 62-76% in de lage lijn. Statistische analyse lieten significante verschillen zien in de groei prestaties van de twee lijnen in de verschillende behandelingen. Lichaamsgewicht bij oogst was het hoogst in testmilieu P200 (123.4 gram in de lage lijn en 131.7 gram in de hoge lijn). De laagste gemiddelde lichaamsgewichten werden gevonden in M50 (92.1 gram) in de lage lijn en 82.4 gram in de hoge lijn (P100). Hoewel de hoge lijn beter presteerde in de meeste test milieus, was er een significante lijn x testmilieu interactie, hetgeen aangeeft dat beide lijnen gevoelig zijn voor het milieu. Er was ook een significante familie x milieu interactie maar alleen voor de lage lijn. De geobserveerde genotype x milieu interactie hield verband met de interactie van specifieke families met de hoeveelheid beschikbare stikstof en zuurstof in de vijvers.

De resultaten beschreven in dit proefschrift laten zien dat er goede perspectieven zijn voor het opzetten van duurzame fokprogrammas voor boeren met weinig economische middelen en zonder dure bijvoedering van eiwitrijke pellets. De vooruitzichten en benodigdheden voor een dergelijk praktisch fokprogramma in economisch minder bedeelde regios van de wereld worden bediscussieerd in hoofdstuk VIII. Bestrijding van de armoede en voedselzekerheid zijn primaire doelen in ontwikkelingslanden, en het is belangrijk dat goedkope fokprogrammas worden opgestart en dat het genetisch verbeterde materiaal beschikbaar voor de plattelandsboeren. Grote genetische variatie is belangrijk voor het succes van een fokprogramma omdat het de respons op korte en langere termijn bepaald. Strategien om de genetische variatie te behouden horen daarom deel uit te maken van een fokprogramma. Fokdoelen dienen samen met lokale boeren opgesteld te worden, bij voorkeur voor elke agro-ecologische zone, zodat elk ras de behoeften van de lokale boeren dekt. Het oprichten van vermeerderings centra waar boeren pootvis kunnen krijgen, of een gedecentraliseerd fokprogramma, moeten ervoor zorgen dat genetisch verbeterd materiaal beschikbaar is wanneer het nodig is. Het in dit proefschrift beschreven fokprogramma zal alleen dan maximaal rendement opleveren als tegelijkertijd boeren worden gestimuleerd om lokaal beschikbare plantaardige restprodukten en dierlijke mest te gebruiken om de natuurlijke produktiviteit van vijvers en daarmee de groei van de genetisch verbeterde vis maximaal te stimuleren.

MUKHTASARI

Kati ya samaki wa aina ya tilapia wanaofugwa kwa chakula, ni Naili tilapia ambaye ni muhimu zaidi duniani, na ndiye tegemeo la wakulima wengi maskini. Wingi wa ufugaji wa Naili tilapia unafanywa kwenye maeneo ya Kitropiki katika mazingira ya kinusu-shadidi kwenye vidimbwi mchanga ambavyo huwekwa mbolea. Wakulima hawa maskini hutumia maliasili zinazotoka shambani, kwa mfano wishwa, ugali kiporo, makapi, majani, na kadhalika. Ili kuongeza mapato ya samaki, inastahili ukuaji wao uende sambamba na wingi wa chakula walacho. Utafiti umeonyesha kwamba kati ya 15-30% ya virutubishi kutoka kwenye chakula cha samaki chenye protini nyingi huvunwa, lakini ni 5-15% pekee ya virutubishi wanavyopata samaki kwenye vidimbwi vinavyowekwa mbolea ambayo huvunwa. Tasnifu hii ni sehemu ya mradi wa INREF-POND (Sura ya Kwanza), inayojumuisha utafiti katika viwango vya samaki, vidimbwi, na mashamba. Lengo la mradi huu ni kutafuta njia ya kuboresha utumiaji wa virutubishi kwenye mashamba yanayotungamanisha ufugaji wa samaki na ufugaji wa wanyama howa na/au kilimo cha mimea. Ili kuongeza uzalishaji wa samaki, tunahitaji kubuni njia za kuboresha utumiaji wa virutubishi kwenye vidimbwi mchanga vinavyotiwa mbolea. Utafiti huu una nia ya (i) kubaini uwezekano wa kutumia uzalishaji teuzi (*selective breeding*) ili kuboresha ukuaji wa Naili tilapia katika hali ya ufugaji yenye pembejeo duni, (ii) kufahamu athari za uzalishaji teuzi juu ya utendaji wa samaki kwa jumla, na (iii) kuchunguza utangamano wa jenotaipu na mazingara ya Naili tilapia.

Ni kawaida kwa wakulima wa tilapia kukuza vifaranga samaki kwenye mahapa-ndani ya-vidimbwi wakitumia vyakula vya protini nyingi vinavyotoka viwandani. Ili kufanya uzalishaji teuzi kwenye mazingara ya lishe duni, inapasa kufahamu mambo yanayochangia ukuaji wa samaki kwenye mazingara haya. **Sura ya Pili** inaonyesha matokeo ya jaribio lililofanywa ili kufahamu hali njema ya kukuza vifaranga samaki vya tilapia kwenye mahapa-ndani ya-vidimbwi. Lengo la masomo haya lilikuwa ni kukadiria jinsi athari za kimazingira na za kijenetiki zinavyochangia ukuaji wa Naili tilapia wanaokuzwa kwenye mahapa-ndani ya-vidimbwi. Kwenye jaribio tangulizi, tulikuza vifaranga samaki ogelezi-juu (*swim-up fry*) na chakula cha samaki cha kijalizo au bila chakula hicho kwenye mahapa yaliyoning'inizwa kwenye vidimbwi vilivyotiwa mbolea. Wingi wa samaki hao ulikuwa ni vifaranga samaki 5, 10, 15 na 20 kwa mita mraba (m^2). Katika jaribio kuu, tulifuga vifaranga samaki ogelezi-juu toka kwa jamii au familia 25, kila jamii ikigawanywa kwenye mahapa manne. Jaribio

lilikuwa kwa muda wa siku 42, na samaki walikuzwa katiko kiwango cha vifaranga samaki 15/m^2. Mahapa yaliyoning'inizwa ndani ya vidimbwi viwili, yalipangwa kwenye safu mbili zilizokuwa mbalimbali, kila safu ikiwa karibu na ufuo wa kidimbwi. Kila siku, vidimbwi vilitiwa mbolea ya kuku. Kisha vifaranga samaki kwenye safu moja kati ya zile mbili kwenye kila kidimbwi walilishwa chakula cha samaki chenye 40% protini mara mbili kwa siku. Matokeo ya jaribio tangulizi yalionyesha kwamba ukuaji wa vifaranga samaki hutegemea wingi wa vifaranga kwa mita mraba na aina ya mlo. Kwenye jaribio kuu, mlo haukuleta tofauti kubwa katika ukuaji wa vifaranga samaki lakini kulikuwa na tofauti kubwa kati ya vidimbwi: uzani wa samaki baada ya siku 42 ulikuwa 1.7 g kwenye kidimbwi cha kwanza (Kidimbwi A) na 0.4 g kwenye kidimbwi cha pili (Kidimbwi B). Kiwezorithi (*heritability*, h^2) cha uzito wa vifaranga samaki kilichokisiwa kutumia data zote na ruwaza ya kigeu-kimoja kilikuwa 0.01±0.06. Makisio ya kiwezorithi kwa kutumia kigeu-jozi yalikuwa 0.59±0.19 kwenye kidimbwi A na 0.05±0.11 kwenye kidimbwi B. Athari za kukua pamoja utotoni zikijumuishwa na za kinasaba/athari za mahapa (c^2) zilikuwa 0.14±0.06 na 0.29±0.07 kwenye kidimbwi A na B, vikifuatana hivyo. Tulipata mahusiano maeneo (*spatial autocorrelation*) makubwa (P = 0.02), kuonyesha kwamba ukuaji wa vifaranga samaki kwenye mahapa jirani ulifanana. Tulipochambua baadhi ya vipengee vya mazingira, tulipata kuwa wingi wa hewa ya okisijeni kwenye maji ulitofautiana sana kati ya vidimbwi. Uhuiano mdogo na hasi wa kijenetiki, r_g, wa kiwango cha -0.27±0.69 wa uzito wa vifaranga samaki kati ya vidimbwi unaelekea kuhusiana na tofauti za wingi wa hewa ya okisijeni kwenye maji na hii inaonyesha kuwepo kwa utangamano wa jenotaipu na mazingira ya Naili tilapia.

Kushindwa kwa tilapia kuvumilia halijoto ya chini huathiri vibaya hali ya uchumi wa wakulima, kwa maana hupunguza muda wa ukuzaji wa samaki hao na huleta mauti ya samaki wakati wa msimu wa baridi kali. Katika **Sura ya Tatu**, uwezo wa kustahimili baridi wa Naili tilapia ulitathminiwa na kiwezorithi cha uwezo wa kustahmili baridi kukadiriwa. Jamii 80 zilifanywa kwa kumpa kila samaki mume majike wawili. Vifaranga samaki walikuzwa kwenye mahapa yaliyoning'inizwa kwenye vidimbwi mchanga vilivyotiwa mbolea ya kuku, na vilikuwa na umri wa siku 41-91 baada ya kutotolewa, urefu wa 50.6 mm, uzito 5.1 g wakati wa kuanza jaribio hilo. Vifaranga samaki walitiwa alama na wakawekwa kwenye chumba baridi. Joto lilipunguzwa kutoka nyuzi 16 centigredi (C) hadi nyuzi 11 C, katika masaa 48, na kutoka nyuzi 11 C hadi nyuzi 8 C, katika kiwango cha nyuzi moja kwa siku.

Tuliueleza uwezo wa kustahimili baridi kama halijoto wakati samaki alipokufa (TAD), na dakika zilizopita na mabadiliko ya halijoto kabla ya kufa kwa samaki (CDH). Kufa kwa samaki wa kwanza kulianza nyuzi 13.6 C na samaki wote wakafa ilipofika nyuzi 8.6 C. Wastani wa TAD ulikuwa nyuzi 10.1 C na CDH ukawa 298.1. Uzito wa samaki uliathiri uwezo wa kustahimili baridi kwa kiwango kikubwa ($P<0.001$). Samaki wadogo (<5 g) waliathirika zaidi kwa halijoto ya chini kuliko samaki wakubwa. Kiwezorithi cha kustahimili baridi kilichokadiriwa kwa ruwaza mnyama kilikuwa 0.08±0.17 kwa CDH, na 0.09±0.19 kwa TAD. Kulikuwa na athari za kukua pamoja utotoni na jamii za 0.33±0.10 kwa CDH na 0.27±0.09 kwa TAD. Viwango hivi vyaonyesha kuwa ukadiriaji wa parameta jenia za kustahimili baridi kwa tilapia unafaa kuhusisha athari nyongezi za kijenetiki na zile za mazingira ya kukua pamoja. Kulingana na matokeo ya jaribio hili, tunahitimisha kuwa njia ifaayo zaidi kuongeza uwezo wa kustahimili baridi kwa vijana wa tilapia ni kwa kuzingatia ufugaji unaoongeza uzito wa samaki kabla ya msimu wa baridi kali.

Athari za jenotaipu, umri, ukubwa, hali-afya (*condition factor*), na mlo (mwani asili au chakula kilichotengenezwa na protini nyingi viwandani) juu ya uwezo wa kustahimili halijoto ya chini wa vijana samaki zilichunguzwa na kuripotiwa kwenye **Sura ya Nne**. Sura hii ililinganisha matokeo ya jaribio lililoripotiwa kwenye **Sura ya Tatu** na jaribio lengine lililofanywa kutambua athari za mazingira na mlo juu ya uwezo wa kustahimili baridi. Kwenye jaribio la kwanza, vijana samaki 775 waliozaliwa na samaki dume 43 na samaki jike 80 walilelewa kwenye halijoto ya juu kwa siku 41-91. Kwenye jaribio la pili, vijana samaki 393 walizalishwa kutoka kwa samaki dume 20 na samaki jike 20, waliochaguliwa kati ya wazazi waliotumika kwenye jaribio la kwanza. Samaki hawa 393 walilelewa kwa siku 42 kwenye halijoto ya chini wakila chakula cha 40% protini au chakula asili cha mwani. Baada ya muda wa kuwakuza, samaki waliwekwa kwenye halijoto iliyopunguzwa polepole. Uwezo wa kustahimili baridi uliathiriwa na jenotaipu, ukubwa, hali-afya, akwariamu ($P = 0.0001$) na mlo ($P = 0.0547$). Umri haukuathiri uwezo wa kustahimili baridi. Samaki waliolelewa kwenye halijoto ya juu walikufa kati ya nyuzi 13.6 na 8.6 C, hali wale waliolelewa kwenye halijoto ya chini walikufa kati ya nyuzi 11.7 na 7.5 C. Hii inaonyesha kuwa ukabilihali wa halijoto ya chini kabla ya kupitia msimu wa baridi unaweza kuboresha uwezo wa kustahimili baridi wa Naili tilapia.

Ufahamu wa kiwezorithi cha ukuaji na mabadiliko shabihi yanayofanyika kwenye nduni nyengine baada ya uzalishaji teuzi unaolenga ukuaji ni muhimu kwa ufanyizi wa miradi bora ya uboreshaji jenia. Katika **Sura ya Tano** na **Sura ya Sita**, zimeripotiwa kadirio za kimaumbile na za kijenetiki za ukuaji, vipimo vya kiwiliwili, nduni za uzao, na urefu wa utumbo zilizorekodiwa kwa vizazi viwili vya uzalishaji teuzi uliolenga ukuaji kwenye pembejeo duni. Mazingira ya uteuzi yalikuwa vidimbwi mchanga vilivyotiwa mbolea ya kuku ya kiwango cha kilo 50/hekta kila siku. Hakuna chakula chochote jalizo kilichopeanwa. Kwa jumla, samaki 6429 wa jaribio walioandikishwa nasaba zao walitumika kwenye uchanganuzi. Kuishi kwa samaki hadi kuvunwa kulitofautiana sana kati ya vidimbwi na kulihududi kati ya 35% na 77%. Kuishi kwa samaki kuliathiriwa na uzito wa samaki ujanani, vidimbwi na umri. Uzito wa samaki wakati wa kuvunwa uliongezeka kutoka wastani wa 67.4 g kwenye kizazi cha mababu (wasioteuliwa) au G_0, mpaka 129.5 g katika G_2. Vizazi vilikuwa tuli (*discrete*) na kwa hivyo parameta za kijenetiki zilikadiriwa kila mwaka pekee (mwane-mwane). Kiwezorithi cha uzito kilihududi kutoka 0.38 mpaka 0.60, na kiwezorithi cha kuishi kilihududi kutoka 0.03 mpaka 0.14. Makadirio ya itikio la uzalishaji teuzi yalikuwa 23.4 g (34.7%) kati ya G_0 na G_1 na 13.0 g (14.9%) kati ya G_1 na G_2 (**Sura ya Tano**). **Sura ya Sita** inaripoti makadirio ya parameta za kimaumbile na za kijenetiki za vipimo vya kiwiliwili, nduni za uzao, na urefu wa utumbo. Makadirio ya kiwezorithi cha vipimo kiwiliwili yalihududi kutoka 0.4-0.6 kwa urefu wa mwili mpaka 0.69-0.79 kwa urefu wa kichwa. Makadirio ya uhuiano wa kimaumbile kati ya uzito na vipimo vya kiwiliwili yalihududi kutoka 0.64 hadi 0.89. Uhuiano wa kijenetiki ulikaribia moja. Kadirio la kiwezorithi cha kukomaa kwa samaki wakati wa kuvunwa lilikuwa 0.13. Viwezorithi vya nduni mwilimfu vilikadiriwa katika G_1 pekee na vilikuwa 0.16 kwa mwilimfu uliotolewa utumbo na 0.06 kwa asilimia-nyama baada ya kutoa matumbo. Uhuiano wa kimaumbile kati ya uzito wa mwili mzima na mwili uliotolewa utumbo ulikuwa 0.84 na uhuiano wa kijenetiki ukawa 0.20. Urefu wa utumbo uliongezeka kila kizazi cha uteuzi. Kiwezorithi cha urefu wa utumbo kilikuwa 0.22. Tena, urefu wa utumbo na uzito wa mwili vilihuiana sana kijenetiki. Matokeo haya yaonyesha yakini kuwa uteuzi uzalishaji wa ukuaji wa Naili tilapia kwenye mazingira ya pembejeo za chini unawezekana.

Athari za mazingira ya uteuzi kwenye utendaji wa samaki walioteuliwa wakati wanapokuzwa kwenye mazingira tofauti yanayotofautiana kwa ushadidi wa utumiaji wa nyenzo ni swala

muhimu la uzalishaji. Kunapokuwa na utangamano kati ya jenotaipu na mazingira, uboreshaji wa kijenetiki upatikanao kwa uteuzi kwenye mazingira fulani huenda usipatikane kwenye mazingira mengine. Katika **Sura ya Saba**, matokeo ya somo lililoundwa kubainisha uwepo wa utangamano kati ya jenotaipu na mazingira katika Naili tilapia yametolewa. Tulilinganisha utendaji wa samaki walioteuliwa kwenye mazingira duni (Samaki Chini) na wale walioteuliwa kwenye vidimbwi vilivyopokea chakula chenye 25% ya protini (Samaki Juu). Samaki hao mbari mbili walijaribishwa kwenye mazingira matano tofauti: kwenye chakula chenye 40% ya protini (P200), chakula chenye 25% ya protini (P100), chakula chenye 16% ya protini (P50), kilo 50 kwa hekta za mbolea ya kuku (M100), au kilo 25 kwa hekta za mbolea ya kuku (M50). Pembejeo za naitrojeni zilifanana kati ya P50 na M50, na kati ya P100 na M100. Kuishi toka kuwekezwa kwenye vidimbwi hadi kuvunwa kwa samaki kulihududi kati ya 70-75% kwa Samaki Juu na kati ya 62-76% kwa Samaki Chini. Uchanganuzi ulionyesha tofauti kubwa za ukuaji kati ya mbari hizo za samaki kwenye mazingira hayo. Wastani wa uzito wa samaki wakati wa mavuno ulikuwa wa juu zaidi kwenye P200 (123.4 g Samaki Chini na 131.7 Samaki Juu). Viwango vya chini vya uzito wastani vilikuwa 92.1 g (mazingira M50) kwa Samaki Chini na 82.4 g (mazingira P100) kwa Samaki Juu. Ijapokuwa Samaki Juu walionyesha utendaji bora kwenye mazingira mengi zaidi, kulikuwa na utengamano mkubwa wa mbari na mazingira, kuonyesha kuwa mbari zote mbili zilikuwa nyetifu kwa mazingira. Utangamano kati ya jamii na mazingira ulipatikana kwenye Samaki Chini pekee. Utangamano huu wa kijenetiki na mazingira ulihusiana na utangamano kati ya jamii za samaki na naitrojeni na pia okisijeni iliyo ndani ya vidimbwi.

Matokeo yaliyoelezwa kwenye tasnifu hii yanaonyesha uwezekano mzuri wa kuanzisha miradi ya uzalishaji wa tilapia kwa minajili ya kilimo kisichohitaji vyakula jalizo ghali vya protini. Uwezekano na mahitaji ya kuanzisha mifumo ya uzalishaji kwa mkulima mdogo kwenye maeneo ya nyenzo duni duniani yamejadiliwa kwenye **Sura ya Nane.** Kwa sababu kuondoa umaskini na kuwa na hali salama ya chakula ni malengo msingi katika nchi zinazoendelea duniani, ni muhimu kwamba mifumo rahisi ya uzalishaji ianzishwe na kudumishwa, na mbegu zilizoboreshwa kijenetiki zifanywe rahisi kupatikana na wakulima wa samaki vijijini. Mwachano mkubwa wa kijenetiki unahitajika katika miradi ya uzalishaji kwa sababu ya umuhimu wa maitikio ya uzalishaji teuzi ya karibuni na ya zama zijazo. Kwa hivyo, mikakati ya kudumisha mwachano wa kijenetiki kizazi baada ya kizazi cha uteuzi

yanafaa kujumuishwa kwenye mpango wa uzalishaji. Malengo muafaka ya mifumo ya uzalishaji yanahitaji kuwekwa tukizingatia kuwahusisha wakulima walengwa, hasa hasa katika kila jimbo au eneo tofauti la kiekolojia la zaraa ili kuhakikisha kwamba mzao wa samaki unatosheleza mahitaji ya mkulima mlengwa mahali popote alipo. Utengenezaji wa vituo vya uzidishaji wa mbegu za samaki ambapo wakulima wanaweza kupata vifaranga samaki au ufanyaji wa miradi ya uzalishaji karibu na wakulima yafaa uhimizwe ili kuhakikisha kwamba mbegu zilizoboreshwa zinawafikia wakulima kwa wakati muafaka. Ili kupata manufaa kamili ya uzalishaji, inahitaji wakulima wahimizwe kufuga samaki wakitumia nyenzo za mashambani mwao na mbolea katika njia zinazofaa ila kuhakikisha kuwa Naili tilapia walioboreshwa kijenetiki wanapata lishe tosha.

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Harrison Charo
10 April 2006

Training and Supervision Plan			**Graduate School WIAS**	
Name PhD student	Harrison Charo Karisa			
Project title	Selection for fast growth in Nile tilapia, *Oreochromis niloticus* (L.) in low-input earthen ponds			
Group	Fish Culture and Fisheries			
Daily supervisor(s)	Dr. Mahmoud Rezk, ICLARM			
	Dr. Henk Bovenhuis; Dr. Hans Komen			
Supervisor(s)	Prof. J.A.J. Verreth; Prof. J.A.M. van Arendonk			
Project term	from February 2002	until February 2006		
Submitted	date: 1 February 2006	first plan / midterm / **certificate**		
EDUCATION AND TRAINING (minimum 21 cp, maximum 42 cp)				
The Basic Package (minimum 2 cp)			year	cp*
WIAS Introduction Course, February 24-27 (mandatory)			2004	1.0
WIAS Course Philosophy of Science and Ethics, March 4- April 1 (mandatory)			2004	1.0
Subtotal Basic Package				**2.0**
Scientific Exposure (conferences, seminars and presentations, minimum 5 cp)			year	cp
International conferences *(minimum 2 cp)*				
ISTA meeting. Manila Phillipines, September 16-19			2004	0.8
World Aquaculture Society (WAS) Conference, 9-13 May			2005	1.0
Larvi 2005, Gent University, Belgium, September 5-8			2005	0.8
Seminars and workshops				
WIAS Science Day, 2002, 2004, 2005			2002/04/05	0.6
Fats and Seafood for Health, WIAS/VLAG Seminar Plus, December 2			2003	0.2
Approaches to sustainable development of animal production, WIAS, October 3			2005	0.1
Presentations *(minimum 4 original presentations of which at least 1 oral, 0.5 cp each)*				
Poster presentation at WIAS Science Day			2004	0.5
Poster presentation at LARVI 2005			2005	0.5
Oral presentation ISTAMeeting Manila, Phillipines, Sept 16-19			2004	0.5
Oral presentation World Aquaculture Society (WAS) Conference, May 9-13			2005	0.5
Subtotal International Exposure				**5.5**
In-Depth Studies (minimum 4 cp)			year	cp
Disciplinary and interdisciplinary courses				
Analysis of Breeding data and Estimation of Genetic parameters, May 12-16			2002	1.0
The biological basis for improved management and selection tools, October 10-14			2005	1.0
QTL detection and fine mapping in complex pedigrees, October 17-21			2005	1.0
PhD students' discussion groups				
Animal Breeding and Genetics Discussion Group				1.0
Undergraduate courses				
Advanced Statistics for Life Sciences			2003/04	4.0
Subtotal In-Depth Studies				**8.0**
Professional Skills Support Courses (minimum 2 cp)			year	cp
The Language Centre PhD Scientific Writing Course, May 4-June 29			2005	1.2
Professional Communication Strategies, 14-15 February			2005	0.8
Subtotal Professional Skills Support Courses				**2.0**
Research Skills Training (apart from carrying out the PhD project, optional)			year	cp
Preparing own PhD research proposal (optional, maximum 4 cp)				4.0
Subtotal Research Skills Training				**4.0**
Didactic Skills Training (optional)			year	cp
Supervising practicals and excursions				
Supervised Kenya and Uganda Trainees in Genetics in Egypt, June 2004			2004	2.0
Supervising MSc theses *(5% of the cp of the thesis)*				
MSc thesis: Evaluation of optimal density for growth of Nile tilapia fry in a low input environment			2002/03	1.3
MSc thesis: Phenotypic and genetic parameters of body and reproductive traits of Nile tilapia			2004/05	1.3
Subtotal Didactic Skills Training				**4.6**
Education and Training Total				**26.1**

*One credit point (cp) equals a study load of approximately 40 hours.

The author was born on 22 February 1972 to William Karisa Yaa and Rachel Kadzo. He was educated at Bamba Full Primary School from 1979 to 1986 and obtained a Kenya Primary School Certificate of Education. He proceeded to Mangu High School from 1987, from where in 1990 he obtained a Kenya Secondary Certificate of Education. In 1992 he joined the Jomo Kenyatta University of Agriculture and Technology (JKUAT) where he graduated with a BSc honours degree in 1996. While studying at JKUAT, he taught Chemistry, Biology and Mathematics at Kilifi Township and Godoma Secondary Schools during the holidays. After his BSc, he worked as a Chemistry and Biology teacher at Sokoke Secondary School from January to June, 1996. In July 1996, he was offered a job at the JKUAT as a Teaching Assistant and at The Kenya Marine and Fisheries Research Institute (KMFRI) as a Research Officer. He opted for the research job where he is currently. In 1999, he obtained two scholarships: a one year MSc. course in Nematology at Gent University, Belgium and an MSc course in Biodiversity at the Swedish Biodiversity Center (CBM) of Uppsala University and the Swedish University of Agricultural Sciences, Uppsala, Sweden. He opted for the MSc. Biodiversity course from which he graduated in June 2001. In October the same year he obtained an INREF-Pond Scholarship to pursue a PhD course in Fish Genetics at Wageningen University.

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