Breeding for improved production of Nile tilapia (*Oreochromis niloticus* L.)

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Chapter I

General introduction

Selective breeding and aquaculture

Selective breeding has been applied successfully to improve production of many livestock species. Especially in the last half century, the ability to distinguish between desirable and undesirable breeding animals has increased and the knowledge to deploy these animals efficiently in breeding programs has been developed. Therefore, dramatic changes in performance in the typical livestock species have been achieved. The genetic potential for milk production of dairy cows in the American Holstein-Friesian population increased e.g. by nearly 3600kg since 1957 (AIPL, 2004). Broiler-chickens grow three times faster to marketable weight on half the amount of food of the vintage broiler since the 1950s (Havenstein et al., 1994). Layer-chickens used in the Netherlands, increased their production of eggs from approximately 41g/day in 1967 to almost 51g/day in 1988 while feed consumption decreased from 115g/day to 112g/day in the same period (Luiting, 1991 and references herein).

The total world production of fish increased marginally from nearly 120 million tons in 1996 to nearly 130 million tons in 2000 (FAO, 2003). In the same period, the proportional contribution of aquaculture to this amount increased by 33% to almost 36 million tons in 2000. The total amount of captures stayed roughly the same at 93 million tons. According to model-studies into future world fish-consumption commissioned by FAO, fish-consumption will increase whereas the total amount of captures will stagnate (FAO, 2003). Their models indicated that the production of fish from aquaculture is expected to increase to 54 million tons in 2030. When this scenario becomes true, the proportional contribution of aquaculture to the total fish-production would increase from 28% in 2000 to 46% in 2030.

Selective breeding in fish is currently in a developing phase compared to breeding in traditional livestock species (Gjerde et al., 2002; Mambrini et al., 2004). At this moment, examples of breeding programs can be found in many species though: e.g. salmon (Refstie, 1990; Martinez et al., 1999; Gjerde et al., 2002), carp (An and Thien, 1993; Bakos and Gorda, 2002; Basavaraju et al., 2003), trout (Kause et al., 2002; Pante et al., 2002; Mambrini et al., 2004) catfish (Waldbieser and Wolters, 1999) and tilapia (Uraiwan and Doyle, 1986; Pullin et al., 1991; Macaranas et al., 1997; Bentsen et al, 1998). However, when FAO's scenario for the future of aquaculture production becomes true, more breeding programs will probably need to be started to contribute to the improvement of aquaculture production efficiency.

Nile tilapia and aquaculture

Nile tilapia has been the fish species with the largest production expansion in recent years. The FAO (2001) indicated that the global tilapia production has increased to 1.1 million tons in 2000. With this expansion, tilapia production is only fractionally lower than the global production of e.g. salmon (1.2 million tons; FAO, 2001). Nowadays, the name "tilapia" is incorrectly used for several fish-species within the genera *Tilapia*, *Sarotherodon* and *Oreochromis*, all belonging to the family *Cichlidae*. The genus *Oreochromis* consists of tropical freshwater fish-species indigenous to Africa of which *O. mossambicus*, *O. aureus* and *O. niloticus* are mostly used in aquaculture production. *Oreochromis niloticus* (Nile tilapia) was introduced in aquaculture around 1965 in Asia because of its capacity to adapt to a wide range of environmental conditions (Bentsen et al., 1998). Around that time, tilapia production was realized mainly by resource-poor fish farmers. Later the species became recognized as candidate for more commercial production. In the mid nineteen-eighties, Langholz (1987) referred to tilapia as "One of the most promising aquaculture candidates worldwide", which indicates that the commercial use of tilapia was not yet established then. In research, an equal trend of expansion could be observed. The occurrence of "tilapia" as title-word in the Aquatic Sciences and Fisheries Abstracts database (ASFA) increased from 17 between 1960 and 1970, to 488, 1079 and 1724 in the following three decades. A large percentage of global tilapia production is realized in (semi) natural environments such as earthen ponds, raceways or in cages or nets in open water. Unlike *O. mossambicus* and *O. aureus*, *O. niloticus* is saline intolerant and needs freshwater. Since tilapia are tropical fish which need water temperatures of at least 15°c., production is mostly realized in (sub)tropical global zones.

In Europe, production systems for Nile tilapia need to be quite different: heating of the water is required and to prevent large losses of energy and waste products, recirculation systems have to be used. To operate cost-effectively, fish densities are mostly higher and pelleted feed is provided. An advantage of production in Europe compared to production in tropical global zones is that the channels to the European market are shorter. Currently, the European market asks for fillets between approximately 100g and 150g because these are considered sufficiently large to serve as a meal for one person. Fillet yield of Nile tilapia depends on e.g. body size and filleting method and varies roughly from 26% to 37% (Rodrigues de Souza and Macedo-Viegas, 2000; Silva et al., 2000) and therefore, fish need to weigh roughly between 700g and 800g.

How to develop a breeding program?

To develop a breeding program, there is general consensus that a number of crucial steps have to be taken (e.g. Cunningham, 1977; Kinghorn, 1983; Bentsen, 1990; Refstie, 1990; Gjerde et al., 2002):

I. A breeding goal has to be defined which states what traits are targeted for genetic improvement, and their (relative) economical importance and desired direction of change. The breeding goal is (mostly) directed by market demands which primary animal production is aiming to satisfy. However, not only traits directly related to production, but also traits that safeguard long term continuity of the breeding program such as fertility and disease resistance can be included in the breeding goal. Furthermore, it is important that breeding goal traits are heritable and that information can actually be measured which is genetically related to the breeding goal traits.

II. A base population of animals has to be available or can be obtained or composed. The base population has to satisfy to two essential criterions: first, the genetic levels for the traits of interest have to be high enough and, secondly, sufficient genetic variation for the traits of interest has to be available. It is obvious that the genetic level guarantees a certain level of production. The genetic variation, however, enables the breeder to select the genetically best animals to serve as parents for the next generation, and thereby increase the genetic level of the population. The larger

the difference between the genetic level of the selected parents and the population mean represented by the genetic variation, the larger the eventual selection response that can be achieved.

III. When steps I and II are completed actual selection can take place. To this end, a selection criterion has to be chosen, and the best animals are selected to serve as parents for the next generation. Especially the number of parents that are selected is critical since this directly influences the rate of inbreeding in the breeding program. A high rate of inbreeding causes a decrease in genetic variance and can compromise long term genetic progress. When breeding programs are started, usually selection takes place within a (pure) line, but when there is evidence which supports the presence of heterosis, or when correlated responses are unfavorable in some traits, crossbred systems can also be opted for.

IV. After step III, the produced offspring are tested, preferably in the same environmental circumstances. After this test, data is collected, and the best of the offspring are selected to serve as parents for the next generation. When the breeding program is reaches step IV for the first time, the data that was collected should be used to estimate genetic parameters and these can, in turn, be used to optimize the breeding program. When step IV is completed, step III is repeated replacing the parents with the selected offspring. In later generations, it is not necessary to estimate genetic parameters each generation. However,

V. During some generations of selection, selection response should be monitored, so that adjustments can be made in the breeding program, in the case a departure from the expected selection response is observed. Departures from expected selection response can occur due to e.g. low accuracy of estimation of genetic parameters or, more seriously, biased genetic parameters due to the use of wrongful estimation models.

Points I to V indicate that genetic parameters are indispensable for the design and optimization of breeding programs. However, genetic parameters for body weight between 700 and 800g are scarce in literature. The work that has been done has mostly concentrated on estimation of genetic parameters of early growth (body weight), i.e. ages below 100 days and body weights below 30g.

Body weight at higher age, has been the subject of only one paper by Langholz (1987), however, feeding was not *ad libitum* in this experiment and the fish only reached 140g in 360 days. Furthermore, fillet yield in fish is generally considered an important trait (Flick et al., 1990; Bosworth et al., 1998; Cibert et al., 1999; Bosworth et al., 2001; Kause et al., 2002). However, only Velasco et al. (1995) has presented genetic parameters for fillet weight in Nile tilapia so far, and no indication of weight or age of their fish was given. Selection for fillet yield in fish raises an additional problem: accurate phenotypic selection criterions for fillet yield in fish lack. When e.g. mass selection programs are targeted, measuring of fillet yield would mean that the animals are sacrificed and that they cannot be used for reproduction anymore.

Breeding programs in Nile tilapia

In literature, there is one well documented example of the development of a breeding program in Nile tilapia. This breeding program was developed within a project that was called the "Genetic Improvement of Farmed Tilapias" (GIFT) project and was initiated by ICLARM/WFC. The project targeted the development of fish production under extensive circumstances in smallholder farms in developing countries by using the latest existing (scientific) knowledge (Pullin et al., 1991; Bentsen et al., 1998). Besides development of new knowledge through research, one of the projects results was a newly composed Nile tilapia strain, based on 4 different wild- and 4 different farmed strains. Selective breeding in 5 consecutive generations resulted in a cumulative genetic improvement of 85% compared to the base population (Dey and Gupta, 2000).

Aim and outline of this thesis

The aim of this thesis was to generate knowledge that supports the design of breeding programs for Nile tilapia targeting genetic improvement of body weight and fillet yield to serve the European market.

In chapter II, four different strains of Nile tilapia are compared using 14 microsatellite markers. These four strains were obtained from various places in the world and in this study, genetic variation within and between strains is investigated. Several statistics are presented to clarify genetic differences on the molecular level between strains and both genetic variation within- and between strains is determined. To enable choices with respect to genetic contributions of

strains for the composition of a base population, the added value of each strains genetic variation to the total set of strains is determined.

In Chapter III, phenotypic characteristics among fillet weight, fillet yield and linear body measurements are investigated in three strains of Nile tilapia. The potential of body measurements such as length-, height- and width of the fish to predict fillet weight and fillet yield is investigated by means of linear regression models. Whether these prediction models could enable mass selection for fillet traits is discussed.

In chapter IV, genetic parameters for body weight, fillet weight, fillet yield and body measurements are estimated. Genetic correlations between traits are calculated and reveal the relationship between body weight and fillet traits. The potential of mass selection on fillet traits is further investigated.

In Chapter V, a random regression model is used to estimate genetic parameters for curves that describe the development of body weight of Nile tilapia in time. Variance components that are continuous in time are estimated and the possibility to change growth patterns in Nile tilapia is discussed. Furthermore, genetic differences between growth patterns of different strains are presented.

In Chapter VI variance components for early body weight were estimated using a model that accounts for genetic effects of competition from tank mates. Whether competitive behavior is genetically determined in Nile tilapia and what the consequences for breeding programs could be, is clarified.

Finally, in chapter VII, the results of this thesis together with various topics related to breeding programs in aquaculture are discussed.

References

An, N.Q., Thien, T.M., 1993. Preliminary results of a silver carp breeding program in Vietnam. Naga, the ICLARM quarterly, 16 (1), 21-23.

Animal Improvement Programs Laboratory (AIPL), 2004. Trend in breeding values for Holstein calculated November 2004. http://aipl.arsusda.gov/dynamic/trend/current/trndHOm.html, accessed 15-11-2004.

Bakos, J., Gorda, S., 1994. Breeding program of Hungarian carp with special regard to intraspecific hybridisation. Proc. 5th World Congr. Genet. Appl. Livest. Prod., Guelph, 7-12 Aug., vol. 19, pp.383-386.

Basavaraju, Y., Penman, D.J., Mair, G.C., 2003. Stock evaluation and development of a breeding program for common carp (*Cyprinus carpio*) in Karnataka, India: Progress of a research project. Naga, the ICLARM quarterly, 26 (2), 30-32.

Bentsen, H.B., 1990. Application of breeding and selection theory on farmed fish. Proc. 4th World Congr. Genet. Appl. Livest. Prod., Edinburgh, 23-27 Jul., vol. XVI, pp.149-158.

Bentsen, H.B., Eknath, A.E., Palada-de Vera, M.S., Danting, J.C., Bolivar, H.L., Reyes, R.A., Dionisio, E.E., Longalong, F.M., Circa, A.V., Tayamen, M.M., Gjerde, B., 1998. Genetic improvement of farmed tilapias: Growth performance in a complete diallel cross expiriment with eight strains of Oreochromis niloticus. Aquaculture 160, 145-173.

Bosworth, B.G., Libey, G.S., Notter, D.R., 1998. Relationships among body weight, body shape, visceral components, and fillet traits in palmetto bass (stripped bass female Morone saxatilis x white bass male m. Chrysops) and paradise bass (stripped bass female m. Saxatilis x yellow bass male m. Mississippiensis). J. World Aquaculture Soc. 29, 40-50.

Bosworth, B.G., Holland, M., Brazil, B.L., 2001. Evaluation of ultrasound imagery and body shape to predict carcass and fillet yield in farm-raised catfish. J. Anim. Sci. 79, 1483-1490.

Cibert, C., Fermon, Y., Vallod, D., Meunier, F.J., 1999. Morphological screening of carp Cyprinus carpio: Relationship between morphology and fillet yield. Aquat. Liv. Res. 12, 1-10.

Cunningham, E.P., 1977.

Dey, M.M., Gupta, M.V., 2000. Socioeconomics of disseminating genetically improved Nile tilapia in Asia: An Introduction. Aquaculture Economics and Management 4, 5-11.

FAO, 2001. Yearbook, Fishery statistics, Aquaculture production. Vol. 92/2. FAO, Rome.

FAO, 2003. Review of the state of world aquaculture. FAO fisheries circular No. 886, Rev 2. FAO, Rome.

Flick, G.J., Barua, M.A., Enriquez, L.G., 1990. Processing finfish. In: Martin, R.E., Flick, G.J. (Eds.), The seafood industry. Van Nostrand Reinholt, New York, pp.117-173.

Gjerde, B., Villanueva, B., Bentsen, H.B., 2002. Opportunities and challenges in designing sustainable fish breeding programs. Proc. 7th World Congr. Genet. Appl. Livest. Prod., Montpellier, 19-23 Aug., Communication no. 06-01.

Havenstein, G.B., Ferket, P.R., Scheidler, S.E., Larson, B.T., 1994. Growth, livability and feed conversion when fed "typical" 1957 and 1991 broiler diets. Poult. Sci. 73, 1785-804.

Kause, A., Ritola, O., Paananen, T., Mantysaari, E., Eskelinen, U., 2002. Coupling body weight and its composition: A quantitative genetic analysis in rainbow trout. Aquaculture 211, 65-79.

Kinghorn, B.P., 1983. A review of quantitative genetics in fish breeding. Aquaculture 31, 283-304.

Langholz, H.-J., 1987. Intraspecific variation in tilapia. Proceedings of the 3rd status seminar, 27 april- 1 may, 1987, Tiberias, Israel. Special publication EAS, no 11, 1990., 103-118.

Luiting, P., 1991. The value of feed consumption data for breeding in laying hens. Doctoral thesis, Department of Animal Breeding, Wageningen Agricultural University, Wageningen, The Netherlands.

Macaranas, J.M., Mather, P.B., Lal, S.N., Vereivalu, T., Lagibalavu, M., Capra, M.F., 1997. Genotype and environment: A comparative evaluation of four tilapia stocks in Fiji. Aquaculture 150, 11-24.

Mambrini, M., Sanchez, M-P., Chevassus, B., Labbe, L., Quillet, E. Boujard, T., 2004. Selection for growth increases feed intake and affects feeding behavior of brown trout. Livest. Prod. Sci. 88, 85-98.

Martinez, V., Neira, R., Gall, G.A.E., 1999. Estimation of genetic parameters from pedigreed populations: lessons from analysis of alevin weight in Coho salmon (*Oncorhynchus kisutch*). Aquaculture 180, 223-236.

Pante, M.J.R., Gjerde, B., McMillan, I., Misztal, I., 2002. Estimation of additive and dominance genetic variances for body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 204, 383-392.

Pullin, R.S.V., Eknath, A.E., Gjedrem, T., Tayamen, M.M., Macaranas, J.M., Abella, T.A., 1991. The genetic improvement of farmed tilapias (GIFT) project: the story so far. Naga, the ICLARM quarterly, 14(2), 3-6.

Refstie, T., 1990. Application of breeding schemes. Aquaculture 85, 163-169.

Rodrigues de Souza, M.L., Macedo-Viegas, E.M., 2000. Effects of filleting methods on processing yield of Nile tilapia (Oreochromis niloticus). Proc. 5th Int. Symp. Tilapia Aquacult., Rio de Janeiro, 3-7 sept, pp. 451-457.

Silva, P.C., Souza, V.L., Cantisani Padua, D.M., Dalacorte, P.C., Goncalves, D.C., 2000. Effect of stocking density on growth and fillet composition of tetra hybrid red tilapia, Israeli strain. Proc. 5th Int. Symp. Tilapia Aquacult., Rio de Janeiro, 3-7 sept, pp. 341-345.

Uraiwan, S., Doyle, R.W., 1986. Replicate variance and the choice of selection procedures for Tilapia (*Oreochromis niloticus*) stock improvement in Thailand. Aquaculture 57, 93-98.

Velasco, R.R., Janagap, C.C., De Vera, M.P., Afan, L.B., Reyes, R.A., Eknath, A.E., 1995. Genetic improvement of farmed tilapias: estimation of heritability of body and carcass traits of Nile tilapia (Oreochromis niloticus). Aquaculture 137, 280-281.

Waldbieser, G.C., Wolters, W.R., 1999. Application of polymorphic microsatellite loci in a channel catfish (*Ictalurus punctatus*) breeding program. J. World Aquaculture Soc. 30, 256-262.

Chapter II

Genetic characterization of four strains of Nile tilapia (*Oreochromis niloticus* L.) using microsatellite markers

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ABSTRACT

Four domesticated strains of Nile tilapia (*Oreochromis niloticus L.*) were genetically characterized using 14 microsatellite markers and 64 animals per strain. Two strains, Chitralada (AIT) and IDRC were obtained from the AIT institute, Bangkok, Thailand. The GIFT strain (5th generation) came from NAGRI, Thailand, and the GÖTT strain was supplied by the University of Göttingen, Germany. The average numbers of alleles per marker were 5.0 (GÖTT), 5.4 (AIT), 5.6 (IDRC) and 7.5 (GIFT). Private alleles were found at all markers with the exception of two. No fixation of alleles was found at any marker. Population differentiation, F_{ST}, was 0.178 (great genetic differentiation) and confirmed grouping of the animals in strains. The expected level of heterozygosity ranged from 0.624 to 0.711, but the observed level of heterozygosity significantly deviated from the expected level in three strains. This was probably due to small population size. Moderate to great genetic differentiation was found between strains. A phylogenetic tree reflected the strains known histories. Application of the Weitzman approach showed that all strains have added value for the total genetic diversity and thus should be retained.

Introduction

Nile tilapia has been the fish species with the greatest production expansion in aquaculture in recent years. Worldwide production of Nile tilapia has increased roughly to 1.1 million metric tons, and is thereby only fractionally lower than the production of e.g. salmon (1.2 million metric tons; FAO, 2001).

For the production cycle, large broodstock populations are mostly maintained in ponds. At regular stages all fish are netted and eggs or fry are collected. Mate selection and mating of the animals is not controlled, and individual fecundity of tilapia is relatively high and highly variable between females (Coward & Bromage, 2000). These factors are generally recognized as the main sources of inbreeding in (fish) breeding populations (Bentsen & Gjerde, 1994; Gjerde et al., 1996; Gjedrem, 1998). In addition, the genetic origin of stocks remains largely unknown (Pullin & Capili, 1988).

Initiatives have been taken to start breeding programs for Nile tilapia. A requirement for a successful breeding program is the presence of sufficient genetic variation in the base population. Genetic variation of currently available domesticated strains has been investigated only occasionally and with varying results (Moreira et al., 2000; Appleyard et al. 2001).

Therefore, the aim of this study was to analyze genetic variation in four domesticated strains of Nile tilapia using microsatellite markers. To accomplish this, the amount of genetic variation and inbreeding levels within strains and genetic differentiation between strains were quantified. The contribution of each strain to the total diversity of the strains was quantified by application of the Weitzman approach (Thaon d'Arnoldi et al., 1998), so that the relative importance of individual strains becomes clear.

Material and methods

Strains

Four domesticated strains of tilapia were available in this study. Fish from the Thai Chitralada strain (AIT), and fish from the International Development Research Centers (IDRC) tilapia project were supplied by the Asian Institute of Technology, Thailand. Fish from the "Genetic Improvement of Farmed Tilapias" (GIFT) project (Eknath et al., 1993) were obtained from the National Aquaculture Genetics Research Institute, Thailand, and were allegedly of the fifth generation. A fourth strain was supplied by the University of Göttingen (GÖTT).

Although the genetic background of strains is often unknown, some relevant details were available. The AIT strain was originally based on a very small sample of fish, probably less than 38 breeders (Tangtrongpiros, 1988). The GIFT strain originates from a cross of four farmed Asian strains and 4 wild African strains of Tilapia (Eknath et al., 1993; Bentsen et al., 1998). Of these crosses, the best performing groups (purebred or crossbred) were selected to form the base population. Subsequently, a combined family and within family selection strategy was adopted for genetic improvement of growth (Dey & Gupta, 2000). Fish of the GÖTT strain were originally imported from Lake Manzala (Egypt) into Stirling (UK), this was around 1983. The initial sample consisted of approximately 40 families, but progeny of 10 families moved to Göttingen. Then, around 1990, descendants of all original 40 families were brought from Stirling to Göttingen, in a second batch, and added to the resident population. Next, each two years 50 males and 50 females were chosen randomly, to produce a new generation. The IDRC strain was set up in a project to genetically improve resident tilapia strains in the Philippines. To accomplish this, at least 12 generations were produced using a within family selection strategy (Bolivar & Newkirk, 2000; Camacho et al., 2001). Bolivar & Newkirk (2000) stated that in 1993 a 10^{th} generation was available.

All strains were imported as fry and kept in closed recirculation systems in the Netherlands. The AIT, IDRC and GIFT strains each consisted of approximately 50,000 larvae and the GÖTT strain consisted of approximately 15,000 larvae. At an approximate body weight of 100 grams, 150 animals per strain were randomly chosen for blood sampling, and 64 of them were randomly chosen for genotyping.

Genotyping

Blood samples were kept in plastic tubes containing Na_2EDTA and stored at -80°c until DNA extraction. Genomic DNA was isolated using the PUREGENE kit (Gentra Systems, Minneapolis, MN, USA), following the manufacturers instructions for non-mammalian animals. The amount of re-hydrated DNA solution was raised to yield final DNA concentrations of 5-10 μg/ml.

Nineteen mostly unlinked microsatellite markers were selected from the database at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Amplification of five markers (*UNH108*, *UNH123*, *UNH169*, *UNH203* and *UNH225*) was not successful in one or more strains and they were excluded from the analysis. Because of overlap in PCR products of three markers (*UNH104*, *UNH178* and *UNH190*), the backward primers were relocated. PCR reactions were performed for 5 min at 95°C, 35 cycles of 30s at annealing temperature (45°C-60°C), and 30s at 72°C, followed by a final elongation step of 4 min at 72°C. Markers were divided over 3 sets. Fragment sizes were calculated relative to the GENESCAN-350 TAMRA marker with with GENESCAN (Perkin-Elmer, Boston, MA, USA). Allele identification was performed using GENOTYPER 2 software (Perkin-Elmer).

Statistical analysis

The total number of alleles and the total number of private alleles were counted for each marker within each strain, and among strains using GENETIX version 4.02 (Belkhir et al., 1998). Overall genetic differentiation of the strains under investigation was evaluated by Wright's F-statistics as proposed by Weir & Cockerham (1984). Observed and expected heterozygosity were calculated per strain and estimates within sub-population fixation of alleles (F_{IS}) were obtained. Significance of F_{IS} was estimated by the Markov chain method (Guo & Thompson, 1992) as implemented in GENEPOP version 3.2a (Raymond & Rousset, 1995). The alternative hypothesis was specified as heterozygote deficit (Rousset & Raymond, 1995).

The length of the Markov chain was 1000 iterations as burn-in period and 100 batches of 2000 iterations after that. Genetic differentiation between strains was quantified using F_{ST} (Weir & Cockerham, 1984) and Rho (Goodman, 1997), which is an estimate of Slatkins (1995) R_{ST} corrected for sample size, with the computer programs GENETIX and RSTCALC version 2.2 (Goodman, 1997). For both F_{ST} and Rho, 95% confidence intervals were constructed by means of 1000 bootstrap samples. A neighbor-joining phylogenetic tree was constructed using Nei's standard genetic distance (D_S) and the program Populations version 1.2.23 (http://www.cnrs-gif.fr/pge). Confidence for the tree was constructed by 10000 bootstrap samples over loci. The relative contribution of each strain to the genetic diversity of the total set was evaluated by the Weitzman approach (1992; 1993; Thaon d'Arnoldi et al., 1998). This approach quantifies the relative loss of genetic diversity caused by the loss of one specific strain, and can be regarded as a measure of uniqueness of individual strains relative to the complete set (WEITZPro Package; Derban et al., 2003).

Results

The average number of alleles per marker varied from 5.0 in the GÖTT strain to 7.5 in the GIFT strain (Table 1). None of the markers showed less than 3 alleles within strains, whereas the maximum number of alleles found was 12 for marker *UNH214* in the GIFT strain. Summarizing over strains, the number of alleles varied from 5 (*UNH146*) to 20 (*UNH211*; Table 1). Private alleles were found at each marker in at least one or more strains with the exception of markers *UNH132* and *UNH231*. The IDRC strain showed the lowest number of private alleles, averaging 0.6 private alleles per marker. Although the average number of alleles per marker of the AIT strain was nearly the lowest (5.4), the average number of private alleles was the highest (1.2). A total number of 4 private alleles were found in the GIFT strain at marker *UNH214*, and multiple occurrences of 3 private alleles were found in different strains and markers (Table 1).

The overall measure of population substructure, F_{ST}, was 0.178 (95% CI: 0.146-0.218), and confirms grouping of the animals in strains. The expected level of heterozygosity over markers, according to Hardy-Weinberg proportions ranged from 0.624 in the AIT strain to 0.711 in the IDRC strain (Table 2). The observed level of heterozygosity significantly deviated from the expected level in three strains: AIT, IDRC and GÖTT (Table 2).

Table 1. Microsatellite marker, corresponding linkage group (lg) number of alleles per marker per strain, number of private alleles per marker per strain (in brackets), number of animals genotyped per marker (superscript) and total number of alleles per marker in all strains.

marker	lg[a]	AIT n=63	GIFT n=64	IDRC n=64	GÖTT n=64	Total
UNH104	1	8 (3) ^{62}	9 (1) ^{64}	6 (0) ^{64}	7 (2) ^{62}	15
UNH106	14	7 (2) ^{61}	10 (0) ^{64}	7 (0) ^{64}	4 (1) ^{62}	13
UNH132	9	5 (0) ^{63}	5 (0) ^{64}	4 (0) ^{59}	3 (0) ^{60}	6
UNH146	4	3 (0) ^{63}	4 (1) ^{64}	3 (0) ^{61}	3 (0) ^{64}	5
UNH149	5	4 (1) ^{62}	7 (2) ^{62}	7 (1) ^{63}	6 (0) ^{58}	11
UNH160	6	6 (0) ^{61}	10 (1) ^{61}	9 (0) ^{61}	7 (0) ^{60}	12
UNH178	9	5 (3) ^{63}	6 (1) ^{64}	4 (0) ^{60}	4 (1) ^{63}	10
UNH190	21	5 (1) ^{61}	6 (0) ^{59}	8 (2) ^{63}	5 (1) ^{60}	11
UNH208	24	7 (3) ^{62}	6 (0) ^{58}	6 (1) ^{63}	4 (0) ^{58}	11
UNH211	30	7 (2) ^{63}	11 (3) ^{64}	10 (2) ^{64}	8 (3) ^{63}	20
UNH212	15	4 (1) ^{31}	7 (1) ^{64}	4 (1) ^{47}	4 (0) ^{62}	9
UNH214	10	5 (0) ^{22}	12 (4) ^{40}	6 (0) ^{21}	6 (1) ^{62}	15
UNH222	2	4 (1) ^{63}	7 (1) ^{63}	4 (1) ^{64}	6 (1) ^{58}	10
UNH231	6	5 (0) ^{59}	5 (0) ^{64}	4 (0) ^{56}	3 (0) ^{63}	6
average		5.4 (1.2) $^{56.9}$	7.5 (1.1) $^{61.1}$	5.6 (0.6) $^{57.9}$	5.0 (0.7) $^{61.1}$	11.0

[a] see: Kocher et al. (1998); Lee et al. (2002)

Table 2. Expected heterozygosity (H_e), observed heterozygosity (H_o), within strain fixation index (F_{IS}), significance of F_{IS} and corresponding standard error (se)

strain	H_e	H_o	F_{IS}	significance (se)
AIT	0.624	0.625	0.007	0.001 (0.001)
GIFT	0.704	0.696	0.019	0.167 (0.012)
IDRC	0.711	0.685	0.045	0.017 (0.002)
GÖTT	0.669	0.612	0.093	0.000 (0.000)

Measures of pair-wise genetic differentiation between strains in Table 3 show that there was moderate to great genetic differentiation between strains. The use of Rho as compared to F_{ST} indicated a relatively higher differentiation between the pairs AIT-GIFT and AIT-GÖTT, but 95% confidence intervals confirmed this only for the pair AIT-GIFT. Both measures indicated

a relatively close relationship between GIFT and IDRC. The marginal loss of genetic diversity after removal of the AIT strain from the total set was on average 50.7% (Table 4). Removal of other strains had a lower impact, but were still considerable (GIFT: -23.6%; IDRC: -20.0%; GÖTT: -32.6%). The ranking of the GIFT and IDRC strains depended on the measure of genetic differentiation used. The phylogenetic tree (Figure 1) clustered the GIFT and IDRC strains, but did not show a further hierarchical structure for the AIT and GÖTT strains. Re-sampling of the tree by means of 10000 bootstraps indicated that the shown branching occurred in 77% of the cases.

Table 3. Between strain estimates of genetic differentiation by F_{ST} (above the diagonal) and Rho (below the diagonal) and 95% confidence intervals constructed by means of 1000 bootstrap samples (between brackets).

	AIT	GIFT	IDRC	GÖTT
AIT	0	0.214 (0.150 - 0.272)	0.217 (0.142 - 0.310)	0.224 (0.163 - 0.280)
GIFT	0.333 (0.283 - 0.390)	0	0.104 (0.079 - 0.132)	0.153 (0.124 - 0.186)
IDRC	0.218 (0.171 - 0.277)	0.084 (0.063 - 0.126)	0	0.178 (0.134 - 0.229)
GÖTT	0.286 (0.234 - 0.347)	0.166 (0.130 - 0.215)	0.142 (0.121 - 0.180)	0

Discussion

Given the characteristics of tilapia reproduction (high and variable fecundity, skewed mating ratios) and small population size, substantial loss of genetic variability is only to be expected when genetic management lacks. Signals of genetic deterioration as reported by Moreira et al. (2000) were not found in this study. Hence, the overall genetic management of these strains seems to have been appropriate with respect to the amount of polymorphisms that have been preserved. Hence, the overall genetic management of these strains seems to have been appropriate with respect to the amount of polymorphisms that have been preserved. The GIFT and GÖTT strains showed the highest and lowest average number of polymorphisms per marker respectively. For the GÖTT strain, this can possibly explained by the relatively low number of animals this population consists of. The GIFT strain is partly based on 4 wild African strains (Eknath et al., 1993). These strains have been reported to show relatively high amounts of polymorphisms (Fuerst et al., 2000). In addition, only five generations have been produced since the base population of GIFT was set up, and the strain has therefore had little chance to lose alleles by random genetic drift.

Table 4. Marginal loss of genetic diversity according to the Weitzman approach[a]

	F_{ST} V(S)=506[b]			Rho V(S)=583			
i	V(S\i)[c]	ΔV=V(S\i)- V(S)	%V=ΔV/ V(S)	V(S\i)	ΔV=V(S\i)- V(S)	%V=ΔV/ V(S)	average (%V)
AIT	282	-224	-44.3	250	-333	-57.1	-50.7
GIFT	402	-104	-20.6	428	-155	-26.6	-23.6
IDRC	377	-129	-25.5	499	-84	-14.4	-20.0
GÖTT	321	-185	-36.6	417	-166	-28.5	-32.6

[a] distance values are multiplied by 1000

[b] V(S) genetic diversity of the total set of strains

[c] V(S\i) genetic diversity of the total set, after removal of strain i

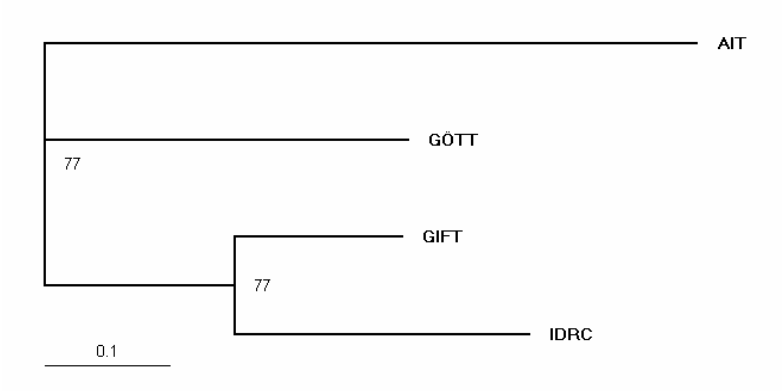

Figure 1. Neighbor-joining phylogenetic tree of four strains of Nile tilapia based on Nei's standard genetic distance (D_S).

Values at the nodes indicate the percentage bootstrap values from 10000 replicates.

Varying inbreeding coefficients (F_{IS}) were found, which were significantly different from zero (α=0.05) in three strains: AIT, IDRC and GÖTT. Since random mating was supposedly practiced to produce the fish for this experiment, the decrease of heterozygosity probably originates from the finite nature of the populations and consequently accumulation of inbreeding. However, even at random mating, when the effective population size (N_e) is small, a decrease of heterozygosity can occur (Crow & Kimura, 1970).

The GIFT breeding program started with high N_e by combining eight different strains, and the subsequent selection program occurred under controlled circumstances. The (random) reproduction round producing larvae for this experiment resulted in genotype proportions in accordance with the Hardy-Weinberg law, so a large increase of inbreeding (or decrease of N_e) was apparently avoided. The opposite was true for the other three strains. The IDRC strain was subjected to a within family selection scheme, which provides a means to prevent fast increases of inbreeding. Accumulation over at least 10 generations has resulted in 4.5% inbreeding, i.e. inbreeding constraints remained within 1% per generation, generally given for breeding programs (Bijma, 2000). In the GÖTT strain, five generations of random mating led to an inbreeding coefficient of 9.3%. Probably, N_e has been small which led to a fast increase of common ancestry. Sampling of individual fish for genotyping was from approximately 15 full-sib families. According to Crow & Kimura (1970) this can cause excess of heterozygosity especially when there are alleles with low frequencies, because of the discreteness of the numbers of possible genotypes. These two opposite effects causing the value of F_{IS}, however, can not be disentangled.

Two measures of genetic differentiation were used in this study, and both indicated moderate to great genetic differentiation. A relatively low value of differentiation between GIFT and IDRC confirms their common genetic background, as both strains were developed from strains resident to the Philippines (Dey & Gupta, 2000; Camacho et al., 2001). Both strains were subjected to selection schemes for at least five (GIFT) and ten (IDRC) generations. However, since the GIFT project started ten years later, around 1990, the ancestral strains must have been retained for some generations. Likewise from the end of both projects ($\pm$1995) until the moment of importation into the Netherlands in 2000. Therefore, the value of F_{ST} found corresponds to at least 20 to 25 generations of genetic differentiation between both strains.

The pair GIFT-IDRC showed moderate genetic differentiation and were clustered as closest neighbors in the phylogenetic tree. The GÖTT and AIT strains were not placed in a

hierarchical way. An introduction of O. niloticus with Egyptian background into the Philippines is mentioned in Pullin (1988), but is dated 1972. A link between the AIT strains background and the other strains lacks entirely in literature. Therefore both strains do not have a direct known genetic link with the GIFT and IDRC strains, which is indicated by the phylogenetic tree. Bootstrap values of 77% indicate moderate to high confidence for this tree.

The result of genetic differentiation for many generations has been the conservation of different genes, and therefore each strain has its own unique genetic 'value'. Application of the Weitzman approach showed that removal of a random strain from the total set of strains, would result in a relative loss of genetic "diversity" between 14% and 57%, depending on the use of F_{ST} or Rho. These amounts are substantial and suggest that all strains should be retained to maintain the amount of genetic diversity present in the total set, although within strain diversity is ignored by this method (Thaon d'Arnoldi et al., 1998).

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References

Appleyard S. A., Renwick J. M. & Mather P. B. (2001) Individual heterozygosity levels and relative growth performance in Oreochromis niloticus (l.) cultured under Fijian conditions. *Aquaculture Research* **32**, 287-96.

Belkhir K., Borsa P., Goudet J., Chikhi L. & Bonhomme F. (1998) GENETIX, logiciel sous WindowsTM pour la genetique des populations. Laboratoire genome et populations, CNRS UPR 9060, Universite de Montpellier II.

Bentsen H. B., Eknath A. E., Palada-de Vera M. S., Danting J. C., Bolivar H. L., Reyes R. A., Dionisio E. E., Longalong F. M., Circa A. V., Tayamen M. M. & Gjerde B. (1998) Genetic improvement of farmed tilapias: Growth performance in a complete diallel cross experiment with eight strains of Oreochromis niloticus. *Aquaculture* **160**, 145-73.

Bentsen H. B. & Gjerde B. (1994) Design of fish breeding programs. *Proceedings of the 5th World Congress on Genetics Applied to Livestock Production* **19**, 353-9.

Bijma, P. (2000) Long-term genetic contributions. Prediction of rates of inbreeding and genetic gain in selected populations. PhD thesis, Animal Breeding and Genetics Group, Wageningen University, The Netherlands, pp 205-6

Bolivar R. B. & Newkirk G. F. (2000) Response to selection for body weight of Nile tilapia (Oreochromis niloticus) in different culture environments. In: *Proceedings from the Fifth International Symposium on Tilapia Aquaculture* **1**, 12-23.

Camacho A. S., Abella T. & Tayamen M. M. (2001) Fish genetics research and development in the Philippines. In: *Fish genetics research in member countries and institutions of the International Network on genetics in Aquaculture, ICLARM conference proceedings* **64**, 71-6.

Coward K. & Bromage N. R. (2000) Reproductive physiology of female tilapia broodstock. *Reviews in Fish Biology and Fisheries* **10**, 1-25.

Crow J. F. & Kimura M. (1970) An introduction to population genetics theory. Harper & Row publishers, New York, Evanston and London.

Derban S., Foulley J.-L. & Ollivier L. (2003) The Weitzpro package. IDDN fr 001040038000, INRA-SGQA, Jouy-en-Josas.

Dey M. M. & Gupta M. V. (2000) Socioeconomics of disseminating genetically improved Nile tilapia in Asia: an introduction. *Aquaculture Economics and Management* **4**, 5-11.

Eknath A. E., Tayamen M. M., Palada-de Vera M. S., Danting J. C., Reyes R. A., Dionisio E. E., Capili J. B., Bolivar H. L., Abella T. A., Circa A. V., Bentsen H. B., Gjerde B., Gjedrem T. & Pullin R. S. V. (1993) Genetic improvement of farmed tilapias: The growth performance of eight strains of oreochromis niloticus tested in different farm environments. *Aquaculture* **111**, 171-88.

FAO yearbook, Fishery statistics, Aquaculture production 2001. Vol 92/2, FAO, Rome.

Fuerst P.A., Mwanja W. W. & Kaufman L. (2000) The genetic history of the introduced Nile tilapia of lake Victoria (Uganda - E. Africa): the population structure of Oreochromis niloticus (Pisces: Cichlidae) revealed by DNA microsatellite markers. In: *Proceedings from the Fifth International Symposium on Tilapia Aquaculture* **1**, 30-40.

Gjedrem T. (1998) Developments in fish breeding and genetics. *Acta Agricultura Scandinavica A suppl.* **28**, 19-26.

Gjerde B., Gjoen H. M. & Villanueva B. (1996) Optimum designs for fish breeding programmes with constained inbreeding - mass selection for a normally distributed trait. *Livestock Production Science* **47**, 59-72.

Goodman S. J. (1997) RST CALC: a collection of computer programs for calculating unbiased estimates of genetic differentiation and determining their significance for microsatellite data. *Molecular Ecology* **6**, 881-5.

Guo S. W. & Thompson E. A. (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* **48**, 361-72.

Kocher T. D., Lee W.-J., Sobolewska H., Penman D. & McAndrew B. (1998) A genetic linkage map of a Cichlid fish, the tilapia (Oreochromis niloticus). *Genetics* **148**, 1225-32.

Lee B.-Y. & Kocher T. D. (2002) Sequencing of BAC library for comparative mapping sex determination genes in tilapia. Proceedings of the international conference on the status of Plant Animal & Microbe Genomes X, http://www.intl-pag.org/10/, last accessed: 25/07/2003.

Moreira H. L. M., Dellagostin O. A. & Erdtmann B. (2000) Levels of inbreeding and relatedness in breeder stocks of Nile tilapia (Oreochromis niloticus) detected by microsatellite analysis. In: *Proceedings from the Fifth International Symposium on Tilapia Aquaculture* **1**, 59-67.

Pullin R.S.V. ed. (1988) Tilapia genetic resources for aquaculture. Proceedings of the workshop on tilapia genetic resources for aquaculture 23-24 March 1987 Bankok, Thailand.

Pullin R. S. V. & Capili J. B. (1988) Genetic improvement of tilapias: problems and perspectives. In: *Proceedings from the Second International Symposium on Tilapia Aquaculture*, 259-66.

Raymond M. & Rousset F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-9.

Rousset F. & Raymond M. (1995) Testing heterozygote excess and deficiency. *Genetics* **140**, 1413-9.

Slatkin M. (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**, 457-62.

Tangtrongpiros M. (1988) The status of wild and cultured tilapia genetic resources in various countries: Thailand. In: *Tilapia genetic resources for aquaculture*, pp. 45-48. ICLARM, Manila, Philippines, Ed. R.S.V. Pullin.

Thaon d'Arnoldi C., Foulley J.-L. & Ollivier L. (1998) An overview of the Weitzman approach to diversity. *Genetics Selection Evolution* **30**, 149-61.

Weir B.S. & Cockerham C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-70.

Weitzman M. (1992) On diversity. *The Quarterly Journal of Economics* **107**, 363-405.

Weitzman M. (1993) What to preserve? An application of diversity theory to crane conservation. *The Quarterly Journal of Economics* **108**, 157-83.

Chapter III

Modeling fillet traits based on body measurements in three Nile tilapia strains (*Oreochromis niloticus* L.)

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ABSTRACT

In Nile tilapia, breeding programs focus mainly on growth, and information on genetic improvement of fillet yield is scarce. In this study, slaughter data were collected on 1215 tilapia and used to analyze the relationship between body measurements and fillet weight and fillet yield. Fish were obtained from three different origins/strains, and raised in a commercial farm in the Netherlands in closed recirculation systems until a final mean weight of 700 grams. Body weight, length, height, width and corrected (=fillet) length were taken prior to slaughter, and used to predict fillet weight and fillet yield using linear regression models.

Average fillet yield was 35.7% with large differences between strains (range 34.4 –38%). There was a strong almost linear relationship between body measurements and fillet weight, but relationships with fillet yield were weak. R^2 of the regression model for fillet weight was 0.95 and the correlation between observed and predicted values of fillet weight 0.98. The effect of strain/origin was significant for each body measurement. The effect of sex and strain*sex was significant for length and corrected (=fillet) length. The fillet yield model explained 15% of the observed variance; the correlation between observed and predicted fillet yield was 0.38, but there were large differences within strains.

We conclude that in Nile tilapia, predicting fillet yield based on body measurements is possible, but correlations can be improved if more accurate methods for measuring body width become available.

Introduction

Fillet yield is regarded as an important trait for the improvement of fish production efficiency (Flick et al, 1990). In Nile tilapia the focus of genetic improvement has been mainly on growth (e.g. Tave and Smitherman,1980; Hulata et al., 1986; Teichert-Coddington and Smitherman, 1988; Bentsen et al., 1998; Eknath et al., 1998; Gall and Bakar, 1999) and studies into the genetic improvement of fillet yield are scarce (e.g. Velasco et al., 1995). Most fish breeding programs are based on mass- or family selection (Bentsen, 1990; Gjedrem, 1992; Bentsen and Gjerde, 1994; Gjedrem, 1998) which complicates selection for fillet yield. To improve fillet yield through these breeding strategies, potentially a number of animals would have to be filleted to provide the trait-information. In the case of family selection, a selection index would have to be designed in which trait information of relatives is used, so identification is necessary.

Studies into alternative strategies for the improvement of fillet yield in fish have mainly concentrated on the use of body measurements as selection-criteria related to fillet yield (e.g. Bosworth et al., 1998; Bosworth et al., 2001; Cibert et al., 1999), but the results from these studies were only moderately positive. One main conclusion has been that the correlation between body weight and fillet weight is generally high and the correlation between body weight and fillet yield is generally low (Bosworth et al., 1998; Cibert et al., 1999). Body measurements of fish have been the traits of interest in these studies mostly because they are descriptive with respect to the shape of an animal. The shape of an animal is not only directly related to its weight, but also has been reported to be related to fillet yield of fish (Bosworth et al., 1998; Cibert et al., 1999; Bosworth et al., 2001).

In tilapia no such studies have been performed whereas body measurements might be characteristics that can be measured on live animals and that could be of value as selection criterion to accomplish correlated response in fillet yield. Therefore, the objective of this paper was to study the relationships between body measurements and fillet weight and fillet yield in three different strains of Nile tilapia. It was investigated whether body measurements are informative for the prediction of fillet traits and a prediction model was developed.

Material and methods

Data

For this study fry of three different Nile tilapia strains were used. Fish from the Chitralada strain (AIT), and fish from the IDRC project were supplied by the Asian Institute of Technology (AIT), Thailand. Fish from the "Genetic Improvement of Farmed Tilapias" (GIFT) project (Eknath et al., 1993) were obtained from the National Aquaculture Genetics Research Institute (NAGRI), Thailand, and were allegedly of the fifth generation. Each strain was raised in a different tank, attached to the same closed recirculation system at densities up to 200 kg/m^3 and feeding was ad libitum.

After one year, the fish had reached an average weight of approximately 700 grams. At six different days, batches of around 200 fish of one strain were randomly sampled for slaughtering, in this way each strain was represented at two days. At the slaughtering facilities, the fish were tagged, body weight (BW) in grams was recorded and the following body measurements were taken (Figure 1): standard length (L), height (H) and head length (HL) using rulers, and width (W) using calipers. To obtain a closer approximation of length of the

fillet, corrected length (CL) was calculated as: CL = L-HL. Scales were removed mechanically and the fish were filleted manually. Each slaughter-day, fish were filleted by two persons (P) and in total, five persons filleted fish in this experiment. Fillet weight (FW, with skin and ribs) in grams was recorded, and fillet yield was calculated as: $F\% = (FW/BW)\cdot 100$. Fish carcasses were opened and gonads were examined to record sex of the individual (S). Fish that lost their tags during the experiment due to the mechanical removal of scales were excluded from the analyses.

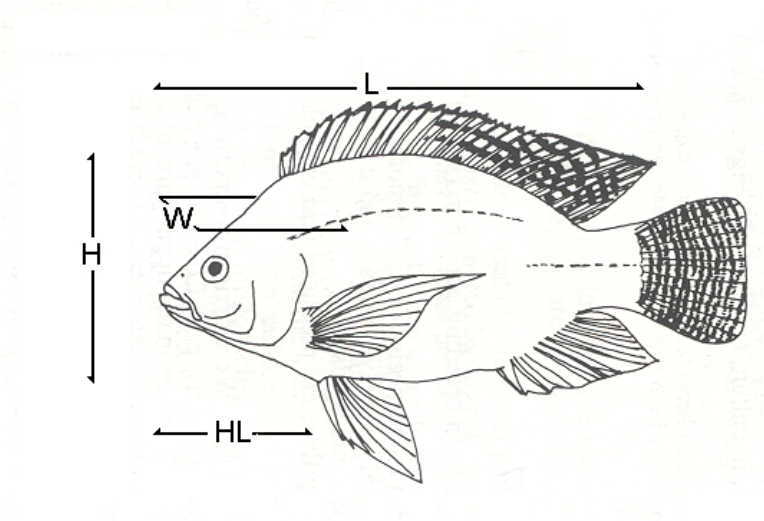

Figure 1. Body measurements taken on each fish: length (L), height (H), width (W) and head length (HL).

Statistical analysis

Mean and standard deviation of body weight, fillet weight, fillet yield and body measurements are presented in Table 1. The effect of person, strain, days nested within strains, sex and body weight on fillet yield, were tested for significance. First order interactions of person*strain, person*body weight, strain*sex, strain*body weight and sex*body weight were also tested. The effect of days nested within strains, the effect of sex and all first order interactions were found to be non-significant. Analysis of variance was performed using proc GLM (SAS institute, 1990) and the least significant factors were removed from the model in a stepwise manner until all remaining effects were significant.

Table 1. Mean and standard deviation (in brackets) of body weight (BW), fillet weight (FW), fillet yield (F%), length (L), height (H), width (W) and corrected length (CL) calculated for all fish, fish of different strains and different sex

		Strain			sex	
	ALL	AIT	IDRC	GIFT	male	female
n	1215	430	414	371	530	685
BW (g)	737 (236)	784 (218)	715 (256)	705 (226)	866 (246)	637 (171)
FW (g)	264 (88)	271 (78)	252 (93)	267 (89)	310 (92)	227 (64)
F%	35.7 (2.7)	34.5 (2.4)	35.2 (2.4)	37.8 (2.2)	35.8 (2.8)	35.7 (2.7)
L (cm)	25.9 (2.6)	26.2 (2.3)	25.5 (2.6)	25.9 (2.9)	27.4 (2.4)	24.7 (2.1)
H (cm)	11.2 (1.4)	11.4 (1.3)	11.0 (1.5)	11.2 (1.5)	11.9 (1.5)	10.6 (1.1)
W (cm)	4.9 (0.5)	4.9 (0.5)	5.0 (0.5)	4.7 (0.5)	5.1 (0.5)	4.7 (0.4)
CL (cm)	17.9 (2.0)	17.9 (1.8)	17.9 (1.9)	18.0 (2.2)	19.0 (1.8)	17.0 (1.6)

The eventual model equation can be written as:

$$y_{ijk} = \mu + P_i + ST_j + \beta * BW_{ijk} + \varepsilon_{ijk} \quad (1)$$

where, y_{ijk} is an observation of F%

μ is the general mean

P_i is a fixed effect of person (i=1,5)

ST_j is a fixed effect of strain (j=1,3)

β is the regression coefficient of body weight

BW is a co-variable of body weight

ε_{ijk} is the random residual term.

Least square means (LSM) were calculated for the fixed effect classes using the LSMEANS option of proc GLM.

To predict fillet yield, data was adjusted for the effect of person but not for the effects of strain and weight, because these could potentially be explained by body measurements. Therefore, LSM of the effect of person from model 1 were used to correct observations of fillet yield. Observations of fillet weight were corrected for the effect of person as:

$FW_C = (F\%_C / 100)BW$. From this point onwards, corrected fillet weight (FW_C) and corrected fillet yield ($F\%_C$) were used in the analyses and will be referred to as fillet weight (FW) and fillet yield (F%).

Pearson correlation coefficients of body measurements, fillet weight and fillet yield were calculated. Furthermore, differences in body measurements between fish were analyzed using an ANOVA model including effects of strain, sex, the interaction of strain and sex and a correction factor for body weight:

$$y_{ijk} = \mu + ST_i + S_j + ST_i * S_j + \beta * BW_{ijk} + \varepsilon_{ijk} \quad (2)$$

where, y_{ijk} is an observation of L, H, W or CL

μ is the general mean

ST_i is a fixed effect of strain (i=1,3)

S_j is a fixed effect of sex (j=male, female)

β is the regression coefficient of body weight

BW is a co-variable of body weight

ε_{ijk} is the random residual term.

For the prediction of fillet weight and fillet yield, simple linear regression models were used including body measurements and body weight as independent variables. For the prediction of fillet yield, a second extended model was used, such that regression coefficients were estimated for body weight and body measurements of each strain. In effect , each regression coefficient from the simple linear model is replaced by three regression coefficients, one for each strain. To test whether parameters were equal for different data-sets, correlations of predicted and observed values of fillet yield were calculated within each strain-class (using parameters estimated in the complete data set).

Results

Fillet yield was normally distributed according to the Shapiro-Wilk test (p=0.098). Analysis of variance of fillet yield revealed that only the effects of strain, person and body weight used in model 1 were significant (p<0.001). One-way interaction effects were non-significant and

eliminated from the model. The effect of strain was relatively big, accounting for a marginal increase of R^2 of 0.21, compared to the effects of person (0.05) and weight (0.01; Table 2). The complete model explained approximately 32% of the observed variance of fillet yield. The general mean (F%) was 35.7%. Least square means of fixed effect classes ranged from 35.0% to 36.8% for the effect of person, and from 34.4% to 38.0% for the strain effect (Table 2). The regression coefficient for body weight on fillet yield was 0.001.

Table 2. Analysis of variance of fillet yield according to model 1: Degrees of freedom (df), marginal (type III) sum of squares (SS) and marginal increase of R^2 as a result of adding an effect to the model

Effect	df	SS (type III)[1]	R^2 increase
Person[2]	4	469.64	0.05
Strain[3]	2	1922.36	0.21
Body Weight	1	70.39	0.01
Error	1207	6212.72	
Model	7	2920.54	0.32

[1] All significant ($p<0.001$);

[2] LSM* person: 34.98^a, 35.08^a, 36.05^b, 36.09^b, 36.84^c;

[3] LSM* strain: 34.4^a (AIT), 35.0^b (IDRC), 38.0^c (GIFT) [* values with different letters differ significantly ($p<0.001$)]

In the analysis of body measurements, the effect of strain was significant on each body measurement. The effect of sex was significant on length, width and corrected length, and significant interaction effects of sex and strain were found on length and corrected length.
Correlations of body measurements and fillet weight (Table3) varied between 0.76 (W) and 0.91 (H), whereas correlations between body measurements and fillet yield varied between -0.02 (H) and 0.19 (W). A scatter plot of length and fillet weight and fillet yield is presented (Figure 2). It confirms that a very strong (approximately linear) relationship exist between length and fillet weight. The relationship between length and fillet yield, however, was very weak. Plots of other body measurements and fillet weight and fillet yield were very similar.

Table 3. Correlation coefficients between body weight (BW), fillet weight (FW), fillet yield (F%), length (L), height (H), width (W) and corrected length (CL)

	FW*	F%*	L	H	W	CL
BW	0.97	0.03	0.93	0.95	0.74	0.87
FW*		0.26	0.90	0.91	0.76	0.87
F%*			0.04	-0.02	0.19	0.11
L				0.87	0.65	0.97
H					0.70	0.82
W						0.63

* FW and F% were adjusted for the effect of person filleting.

Table 4. Fillet weight model: Intercept (μ), regression coefficients (b), goodness of fit (R^2), the correlation coefficient (r ALL) between observed and predicted values, and correlation coefficients (r) between observed and predicted values calculated for the AIT, GIFT and IDRC strains

parameter	estimate
μ	4.19
$b_{(BW)}$	0.38
$b_{(L)}$	-8.61
$b_{(H)}$	-6.089
$b_{(W)}$	13.02
$b_{(CL)}$	11.71
R^2	0.95
r (ALL)	0.98
r (within AIT)	0.97
r (within GIFT)	0.98
r (within IDRC)	0.98

Table 5. Fillet yield model: Intercept (μ), regression coefficients (b), goodness of fit (R^2), the correlation coefficient (r ALL) between observed and predicted values, and correlation coefficients (r) between observed and predicted values calculated for the AIT, GIFT and IDRC strains

parameter	estimate
μ	32.44
$b_{(BW)}$	ns[1]
$b_{(L)}$	-0.94
$b_{(H)}$	-0.56
$b_{(W)}$	1.86
$b_{(CL)}$	1.39
R^2	0.15
r (ALL)	0.38
r (within AIT)	0.30
r (within GIFT)	0.19
r (within IDRC)	0.09

[1] non-significant

R^2 of the fillet weight model was 0.95 (Table 4), consequently the correlation between observed and predicted values of fillet weight resulting from the model was 0.98. Body weight and all body measurements were significant ($p<0.001$). Use of parameters based on the complete data-

set gave accurate predictions of fillet weight in separate strains: in the AIT strain the correlation between observed and predicted fillet weight was 0.97, in the GIFT strain it was 0.98 and 0.98 in the IDRC strain (Table 4).

The fillet yield model explained 15% of the observed variance (Table 5), therefore the correlation between observed and predicted values of fillet yield resulting from the model was 0.38. All body measurements were significant in the model ($p<0.001$), however, body weight was not significant and thus removed from the model. Use of parameters based on the complete data-set did not give accurate predictions within separate strains: in the AIT strain the correlation was 0.30, in the GIFT strain 0.19 and 0.09 in the IDRC strain (Table 5).

Use of separate regression coefficients for each body measurement of each strain, gave an improvement of the explanatory value of the model: R^2 increased from 0.15 to 0.35. Use of these parameters for prediction of fillet yield in separate strains resulted in correlations between predicted and observed fillet yield of 0.35, 0.21 and 0.16, for the AIT, GIFT and IDRC strains respectively (Table 6).

Table 6. Extended fillet yield model: Intercept (μ), regression coefficients (b), goodness of fit (R^2), the correlation coefficient (r) between observed and predicted values (ALL), and correlation coefficients (r) between observed and predicted values calculated for the AIT, GIFT and IDRC strains

parameter	estimate			
	ALL	AIT	GIFT	IDRC
μ	35.76			
$b_{(BW)}$		0.005	0.000	0.003
$b_{(L)}$		-0.61	-0.32	-0.18
$b_{(H)}$		-0.92	-0.31	-0.43
$b_{(W)}$		0.78	0.51	0.79
$b_{(CL)}$		0.99	0.60	0.17
R^2	0.35			
r	0.59	0.35	0.21	0.16

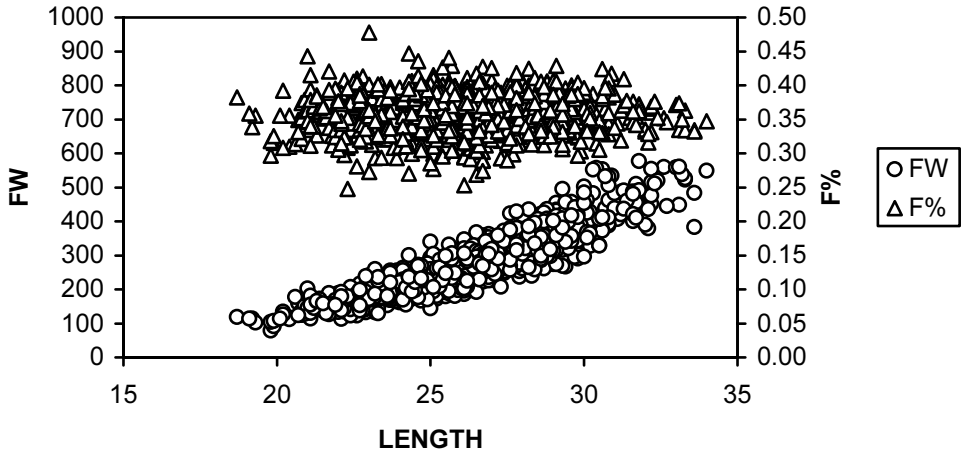

Figure 2. Scatter plot of length and fillet weight (FW) and fillet yield (F%) both adjusted for the effect of person filleting.

Discussion

Fillet yield in Nile tilapia has not been the subject of many studies. Fillet yields were reported, ranging from 26% to 37%, depending on the size of the fish and the filleting method (e.g. Rodrigues de Souza and Macedo-Viegas, 2000; Silva et al., 2000). Average fillet yield in the current study was 35.7%, which is relatively high. Relationships of fillet yield on body weight have been mentioned earlier in Nile tilapia (e.g. Rodrigues de Souza and Macedo-Viegas, 2000; Silva et al., 2000) as well as in other species; e.g. bass (Bosworth et al., 1998), carp (Cibert et al., 1999) and rainbow trout (Kause et al., 2002). In the current study a significant relationship between fillet yield and body weight could be confirmed, however, only a small part of the observed variance of fillet yield could be explained. Rearing fish to higher weights as a strategy to increase fillet yield, suggested e.g. in carp by Cibert et al. (1999) and in bass by Bosworth et al. (1998) can theoretically be functional in Nile tilapia. However, since the regression coefficient of body weight on fillet yield was only 0.001, i.e. by extrapolation, each extra 100 grams of body weight is predicted to result in 0.1% extra fillet yield.

The effect of person was of considerable influence on fillet yield, with a difference of nearly 2% between LSMs of the worst and best filleters and a marginal increase of R^2 of 5%. This indicates that selection and/or training of filleters could be used to improve profitability of fish processing, especially when keeping in mind that the improvement of fillet yield at animal level is complex. The largest portion of variance of fillet yield was explained by the effect of strain. The difference in LSMs was around 3.6% between the worst and best strains and inclusion of

this effect in the model caused a marginal increase of R^2 of 21 %, although modeling of fillet yield showed that the effect of strain is confounded with body measurements. Other than body shape, some reasons can be thought of as a cause for fillet yield differences of strains: e.g. different environmental conditions during incubation or larval stage, genetic differences, tank effects etc. However, the structure of the current data did not allow us to disentangle these effects.

Correlations between body measurements and fillet weight were all high. Prediction of fillet weight with body measurements was therefore successful. Correlations between body measurements and fillet yield were non-significant with the exception of width and corrected length. In addition, the correlation between width and fillet yield was almost twice the magnitude of the correlation between corrected length and fillet yield. Of all body measurements width has clearly the best potential to serve as predictor of fillet yield, although it could not be measured as accurately as other body measurements. One has to make sure that the jaws of the calipers are long enough to reach the widest point of the fish, and that the pressure of the jaws on the body of the fish is equal with each measurement. With a more accurate measurement, the measurement-error can be lowered and this could result in a higher correlation between fillet yield and width of the fish.

With the analysis of body measurements it was shown that significant differences were present between strains, sexes or between the interaction of these factors. The prediction model for fillet yield improved substantially after using separate regression coefficients for body measurements of each strain, even when taking the number of parameters to be estimated into account (Akaikes information criterion: AIC=-2847.33 vs. AIC=-2681.45). Still, the best prediction model for fillet yield explained 0.35% of the observed variance, which implies a correlation of 0.59 between predicted and observed values of fillet yield. Note, that this value was achieved when using all data. Within strains, as would probably be more realistic in practice, the correlations were lower and on average 0.25. When parameters were estimated within separate strains, R^2s did not improve substantially. Alternative traits like ratios of body measurements or the use of non-linear models did not improve the results either.

Improvement of fillet yield via correlated response from selection on body measurements still appears to be hard. Also, the variation of the trait is relatively low. So, questions that remain are whether this model could already be used or whether it is necessary and/or worthwhile to try to develop a more accurate model to predict fillet yield of fish for selection purposes. In other

words: what is the difference in response to selection using the current model or e.g. using a hypothetical more accurate model? Response from selection on a correlated trait depends on the heritabilities of the true and the modeled traits and the genetic correlation between them (Falconer and Mackay, 1996). When heritabilities are assumed equal for the modeled and the true trait, and genetic correlations are assumed equal to phenotypic correlations, the relative difference of these correlations indicates the relative improvement of genetic response using a more accurate model. So, a correlation between predicted and observed fillet yield of say, 0.8, would increase the response by 0.8/0.25=3.2 times as compared to the current situation where the correlation is 0.25 on average.

Until that time, selection for fillet weight seems a good alternative because it is proved to be possible and can be a logical choice in a breeding program since it is a combination of body weight and fillet yield. Also, it is directly related to the trait which determines a producers income.

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References

Bentsen, H.B., 1990. Application of breeding and selection theory on farmed fish. Proc. 4th World Congr. Genet. Appl. Livest. Prod., Edinburgh, 23-27 Jul., vol. XVI, pp.149-158.

Bentsen, H.B., Gjerde, B., 1994. Design of fish breeding programs. Proc. 5th World Congr. Genet. Appl. Livest. Prod., Guelph, 7-12 Aug., vol 19, pp. 353-359.

Bentsen, H.B., Eknath, A.E., Palada-de Vera, M.S., Danting, J.C., Bolivar, H.L., Reyes, R.A., Dionisio, E.E., Longalong, F.M., Circa, A.V., Tayamen, M.M., Gjerde, B., 1998. Genetic improvement of farmed tilapias: Growth performance in a complete diallel cross expiriment with eight strains of Oreochromis niloticus. Aquaculture 160, 145-173.

Bosworth, B.G., Libey, G.S., Notter, D.R., 1998. Relationships among body weight, body shape, visceral components, and fillet traits in palmetto bass (stripped bass female Morone saxatilis x white bass male m.

Chrysops) and paradise bass (stripped bass female m. Saxatilis x yellow bass male m. Mississippiensis). J. World Aquaculture Soc. 29, 40-50.

Bosworth, B.G., Holland, M., Brazil, B.L., 2001. Evaluation of ultrasound imagery and body shape to predict carcass and fillet yield in farm-raised catfish. J. Anim. Sci. 79, 1483-1490.

Cibert, C., Fermon, Y., Vallod, D., Meunier, F.J., 1999. Morphological screening of carp Cyprinus carpio: Relationship between morphology and fillet yield. Aquat. Liv. Res. 12, 1-10.

Eknath, A.E., Tayamen, M.M., Palada-de Vera, M.S., Danting, J.C., Reyes, R.A., Dionisio, E.E., Capili, J.B., Bolivar, H.L., Abella, T.A., Circa, A.V., Bentsen, H.B., Gjerde, B., Gjedrem, T., Pullin, R.S.V., 1993. Genetic improvement of farmed tilapias: The growth performance of eight strains of Oreochromis niloticus tested in different farm environments. Aquaculture 111, 171-188.

Eknath, A.E., Dey, M.M., Rye, M., Gjerde, B., Abella, T.A., Sevilleja, R., Tayamen, M.M., Reyes, R.A., Bentsen, H.B., 1998. Selective breeding of Nile tilapia for Asia. Proc. 6th World Congr. Genet. Appl. Livest. Prod. 27, 89-96.

Falconer, D.S., MacKay, T.F.C., 1996. Introduction to quantitative genetics. 4th Ed. Longmand Sci. and Tech., Harlow, UK.

Flick, G.J., Barua, M.A., Enriquez, L.G., 1990. Processing finfish. In: Martin, R.E., Flick, G.J. (Eds.), The seafood industry. Van Nostrand Reinholt, New York, pp.117-173.

Gall, G.A.E., Bakar, Y., 1999. Stocking density and tank size in the design of breed improvement programs for body size of tilapia. Aquaculture 173, 197-205.

Gjedrem, T., 1992. Breeding plans for rainbow trout. Aquaculture 100, 73-83.

Gjedrem, T., 1998. Developments in fish breeding and genetics. Acta Agric. Scand. section A suppl. 28, 19-26.

Hulata, G., Wohlfarth, G., Halevy, A., 1986. Mass selection for growth rate in the Nile tilapia (Oreochromis niloticus). Aquaculture 57, 177-184.

Kause, A., Ritola, O., Paananen, T., Mantysaari, E., Eskelinen, U., 2002. Coupling body weight and its composition: A quantitative genetic analysis in rainbow trout. Aquaculture 211, 65-79.

Rodrigues de Souza, M.L., Macedo-Viegas, E.M., 2000. Effects of filleting methods on processing yield of Nile tilapia (Oreochromis niloticus). Proc. 5th Int. Symp. Tilapia Aquacult., Rio de Janeiro, 3-7 sept, pp. 451-457.

SAS Institute Inc. SAS® User's Guide: Statistics, Version 6 Edition. Cary, NC: SAS Institute Inc., 1990.

Silva, P.C., Souza, V.L., Cantisani Padua, D.M., Dalacorte, P.C., Goncalves, D.C., 2000. Effect of stocking density on growth and fillet composition of tetra hybrid red tilapia, Israeli strain. Proc. 5th Int. Symp. Tilapia Aquacult., Rio de Janeiro, 3-7 sept, pp. 341-345.

Tave, D., Smitherman, R.O., 1980. Predicted response to selection for early growth in tilapia Nilotica. Trans. Am. Fish. Soc. 109, 439-445.

Teichert-Coddington, D.R., Smitherman, R.O., 1988. Lack of response by tilapia Nilotica to mass selection for rapid early growth. Trans. Am. Fish. Soc. 117, 297-300.

Velasco, R.R., Janagap, C.C., De Vera, M.P., Afan, L.B., Reyes, R.A., Eknath, A.E., 1995. Genetic improvement of farmed tilapias: estimation of heritability of body and carcass traits of Nile tilapia (Oreochromis niloticus). Aquaculture 137, 280-281.

Chapter IV

Genetic parameters for fillet traits and body measurements in Nile tilapia (*Oreochromis niloticus* L.)

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ABSTRACT

Fillet weight is an economically important trait in Nile tilapia production for the European market which asks for fish with average body weights of at least 700 grams. Genetic parameters to design or optimize breeding programs for these body weights are lacking. In an earlier study we showed that high phenotypic correlations exist between body measurements and fillet weight and low phenotypic correlations between body measurements and fillet yield. To evaluate the potential of mass selection for fillet traits however, genetic parameters are required. The aim of the current study was to estimate genetic parameters for body weight, fillet weight, fillet yield and body measurements. Therefore, slaughter data was collected on 1884 pedigreed Nile tilapias. Measurements of body weight, length, width, corrected (=fillet) length and head length were taken on each fish. Filleting machines were used to fillet fish and measurements of fillet weight, fillet yield and head weight were collected. Subsequently, genetic parameters were estimated. Heritabilities were 0.26 for body weight, 0.24 for fillet weight and 0.12 for fillet yield. The genetic correlation between body weight and fillet weight was 0.99 and between body weight and fillet yield 0.74. The genetic correlation between fillet weight and fillet yield was 0.81. Genetic correlations between fillet weight and body measurements were 0.89 for length, 0.70 for head length, 0.94 for width and 0.91 for corrected length. Genetic correlations between fillet yield and body measurements were 0.62 for length, 0.47 for head length, 0.98 for width and 0.60 for corrected length. The potential of mass selection aiming to improve fillet weight was evaluated by a number of selection indexes. The accuracy of a selection index including only body weight indicated that in this way almost the same amount of the selection response can be achieved compared to what hypothetical direct selection for fillet weight would. The use of only width in the selection index would result in 8.5% lower selection response than the use of body weight. We conclude that body weight is the best predictor for fillet weight compared to body measurements.

Introduction

On the European market, Nile tilapia producers are paid for the weight of fillets and fillet traits are thus very important. Studies into improvement of fillet traits in fish have mostly focused on selection strategies where correlated traits are used (Bosworth et al., 1998, 2001; Cibert et al., 1999; Rutten et al., 2004a). This selection strategy is attractive because selection candidates do not have to be sacrificed. When traits correlated to fillet can be identified, genetic improvement can be generated through correlated response. More complicated selection strategies with e.g.

tagging or sib-testing are very labor-intensive and costly and often require extensive facilities and are therefore less attractive. In Nile tilapia, we investigated correlations between body measurements and fillet traits on the phenotypic level (Rutten et al., 2004a). In that study, the correlation between body measurements and fillet weight was generally high while the correlation between body measurements and fillet yield was generally low. This has been observed in other fish species as well (Bosworth et al., 1998, 2001; Cibert et al., 1999). However, to analyze the potential for mass selection on fillet traits, knowledge on genetic parameters is required.

Mass selection requires a relation between the phenotype of an animal and the genotype, i.e. a significant heritability. To our knowledge, heritabilities for fillet weight in Nile tilapia were only published once (h^2_s males=0.63, h^2_s females=0.30; Velasco et al., 1995) and genetic correlations between fillet traits and body measurements are not available. Genetic parameters enable calculation of expected selection response and evaluation of alternative information sources. Moreover, they enable clarification whether investments into e.g. strategies with tagging of fish can be cost effective in terms of extra genetic gain generated in the breeding program.

The aim of this study was to estimate genetic parameters for fillet traits and related body measurements in Nile tilapia, and to provide insight into the effectiveness of mass selection for fillet traits based on body measurements.

Material and methods

Experiment

Seventy-three full-sib families were produced by parents originating from four different strains of Nile tilapia (*Oreochromis niloticus*; for details on strains see: Rutten et al., 2004a, 2004b). Because of initial female fertility problems probably due to transportation of the fish, each female was crossed with two males. In this way the number of contributing parents and the number of crosses could be maximized. As a result of this strategy, pure line matings as well as crosses between strains and paternal- as well as maternal half-sib families were present. Eventually, 40 dams and 56 sires contributed to the full-sib families.

One hundred and fifty fry of each full-sib family were reared in separate tanks (25 l) under commercial circumstances in a closed recirculation system. When the fry reached an average weight of approximately 5 grams, 100 fry per family were individually tagged using Floy® fingerling tags. In total, 7300 animals were tagged. Due to the fact that spawning of females

occurred unsynchronized, families were produced over a period of 12 weeks. The first 35 families were tagged and combined into one tank (4500 l). Later, the remaining 38 families were tagged and also combined in one tank. At the moment of combining, randomly chosen untagged fish were added to the tank, to increase the stocking density to commercial levels (5000 fish in 4500 l). The first 35 families were produced by 24 dams and 35 sires and the second 38 families by 27 dams and 34 sires. In total, 11 dams and 13 sires had progeny in the first and second group of families. In this way confounding of effects of environment and group were avoided.

During the rearing period each fish was weighed five times. After measurement 2, the fish from each tank were split up into 2 tanks (4500 l) to decrease the stocking density. This was organized in such a manner that half of the members of one family were placed in one tank randomly, whereas the other half was placed in the other tank. To decrease the stocking density in the last stage (after measurement 4), two 4500 l tanks were combined again into one tank of 20000 l, so that families were joined again as in the original configuration. In this way each family was represented equally in each tank (for more details see: Rutten et al, 2005). Because of severe tag loss, 3076 Floy® tags were replaced with PIT tags during the experiment. Based on the last measurement of weight, breeding values for body weight were estimated, and the best 200 animals were selected (±6.5%). A random sample from the remaining 93.5% (=1884 fish) was slaughtered for the current experiment.

Fish were slaughtered in four batches, on four consecutive days. Before slaughtering fish were weighed (BW in grams) and the following body measurements were taken on each fish (in cm): body weight (BW), standard length (L), width of the fish (W), length of the head (HL), corrected length (CL=L-HL; Rutten et al., 2004a). Width of the fish was measured using two vertical plates perpendicular to each other which could be moved to close in a fish. The distance between the two plates was measured using calipers (Rutten et al., 2004a).

The filleting process was as follows: first, scales were mechanically removed; then, fish were decapitated mechanically and gutted by hand; finally, fish were filleted using a filleting machine. Fillets included skin and ribs. During this process, the weight of the head (HW) and the weight of two fillets together (FW) were measured. Head- and fillet yield were calculated as H%=(HW/BW)×100 and F%=(FW/BW)×100 respectively. Sex of the fish was recorded during the rearing phase of the experiment, when tags were replaced.

Data Analysis

The data was corrected for fixed effects of sex, tank (during the experiment a maximum of 4 tanks was used to rear the fish), slaughter day and age of the fish. Random effects in the model included additive genetic- and (full-sib) family effect following recommendations of Martinez et al. (1999) and Pante et al. (2002). The random family effect can comprise non-additive genetic, common environmental and maternal genetic effects. Genetic groups were included in the model to correct for different genetic means of different strains (Westell et al., 1988; Quaas, 1988). In matrix notation the model can be written as:

$$y=Xb+ZQg+Zu^*+Wf+\varepsilon$$

where, X is an incidence matrix for the fixed effects vector b (including a covariate for age), g is a vector with genetic group means, u^*, f and ε are vectors with random additive genetic-, family- and residual effects, Z and W are incidence matrices and Q is an incidence matrix relating base animals to their genetic group means. The model has expectations and variance:

$$E(y)=Xb \text{ and } E(u^*)=E(f)=E(\varepsilon)=0$$

$$V(y)=ZAZ'\sigma^2_{u^*}+WIW'\sigma^2_f+I\sigma^2_\varepsilon.$$

where A is the numerator relationship matrix and I is an identity matrix. Estimated animal additive genetic effects can be written as $\hat{u}=Q\hat{g}+\hat{u}^*$ (Quaas, 1988).

Heritabilities were estimated using a bivariate setting of the model. The first trait in the analysis was always the fifth measurement of body weight, i.e. the trait of selection, since this was available for all animals. The second trait was one of the traits measured during the filleting experiment. Genetic correlations were estimated using a trivariate setting of the model. The first trait in the analysis was always the fifth measurement of body weight again, and the second and third traits were traits measured during the filleting experiment. In this way, a non-selected trait was present in the data structure always which prevents biased variance estimates from selected data (Ouweltjes et al., 1988).

Variance ratios $h^2=\sigma^2_{u^*}/\sigma^2_p$ (where $\sigma^2_p=\sigma^2_{u^*}+\sigma^2_f+\sigma^2_\varepsilon$) and $c^2=\sigma^2_f/\sigma^2_p$ were calculated per trait, and phenotypic and genetic correlations between all traits were calculated as the covariance divided by the product of the standard deviations per trait: $r=\sigma_{1,2}/(\sigma_1\times\sigma_2)$. Solutions to the model were generated using restricted maximum likelihood implemented in ASReml software (version 1.10, 15 oct 2003; Gilmour et al., 2002).

Estimated genetic parameters were used to evaluate the potential of mass selection for fillet weight. Selection index theory provided the framework for the design of a number of indexes.

The computer program SelAction (Rutten et al., 2002) was used to calculate accuracies of a number of selection indexes to clarify the added value of body measurements.

Results

Means and standard deviations from raw data for all traits are presented in Table 1. The fish used in this experiment were 426 days of age on average, with a standard deviation of 21.8 days. Body weight was 787.7 grams with a standard deviation of 313.1 grams. Standard-length of the fish was 25.9 cm on average, which was divided in 8.0 cm for the head, and 17.9 cm for the posterior part of the body, excluding the tail fin. Head weight was 192.5 grams on average, which was 25.2 % (H%)of the total body weight. Fillet weight was 308.1 grams on average, which was 37.3% (F%) of the total body weight. Coefficients of variation indicated that all weight-based traits (BW, HW and FW) had relatively large variation (0.344-0.495) compared to other traits (0.135-0.179).

Genetic parameters are presented in Table 2. The heritabilities ranged from 0.12 (H% and F%) to 0.27 (HL). The heritability of fillet yield was 0.12, and that of fillet weight 0.24. Standard errors of heritabilities were relatively large, but heritabilities were significantly different from zero for body weight, length head length, fillet weight and fillet yield. The ratio family-/phenotypic variance (c^2) ranged from 0.02 (F%) to 0.07 (L, W, CL, HW), standard errors, however, were relatively large.

Phenotypic- and genetic correlations are presented in Table 3. Phenotypic correlations between body weight and body measurements ranged from 0.80 (HL) to 0.90 (W). Phenotypic correlations between body measurements and fillet weight ranged from 0.72 (HL) to 0.86 (W). The phenotypic correlation between body weight and fillet weight was 0.95, and between body weight and fillet yield it was 0.48. The phenotypic correlation between body measurements and fillet yield ranged from 0.38 (HL) to 0.51 (W).

Genetic correlations were mostly larger than phenotypic correlations. The genetic correlations between body weight and body measurements were all between 0.76 and 0.92. Among body measurements, the genetic correlation ranged from 0.55 to 0.98, however, the genetic correlation ranged from 0.82 to 0.98 when head length was ignored. Between body weight and fillet weight, the genetic correlation was 0.99, and the genetic correlation between body weight and fillet yield was 0.74. The genetic correlation between fillet weight and fillet yield was 0.81.

Note, that all body measurements with the exception of head length, had a larger genetic correlation with fillet weight than with body weight.

Table 1. Mean, standard deviation and coefficient of variation (CV) from raw data for body weight (BW), length (L), head length (HL), width (W), corrected length (CL=L-HL), head weight (HW), fillet weight (FW), head percentage (H%) and fillet percentage (F%) of all fish used in the experiment.

Trait	μ	σ	CV
BW (g)	787.7	313.1	0.397
L (cm)	25.9	3.5	0.135
HL (cm)	8.0	1.1	0.138
W (cm)	5.1	0.9	0.176
CL (cm)	17.9	2.5	0.140
HW (g)	192.5	66.3	0.344
FW (g)	308.1	152.5	0.495
H% (%)	25.2	4.5	0.179
F% (%)	37.3	5.8	0.155

Table 2. Estimated phenotypic variance (σ^2_p) and the additive genetic- (h^2) and family variance (c^2) as a proportion of the phenotypic variance (standard errors in brackets) for all traits.

Trait*	σ^2_p	h^2	c^2
BW	45454.8	0.26 (0.12)	0.06 (0.05)
L	5.21	0.25 (0.12)	0.07 (0.05)
HL	0.56	0.27 (0.12)	0.05 (0.04)
W	0.39	0.25 (0.13)	0.07 (0.05)
CL	2.90	0.19 (0.12)	0.07 (0.05)
HW	2269.1	0.15 (0.09)	0.07 (0.04)
FW	11406.3	0.24 (0.11)	0.06 (0.04)
H%	15.13	0.12 (0.07)	0.02 (0.03)
F%	24.01	0.12 (0.06)	0.02 (0.02)

* for legend see Table 1

Table 3. Phenotypic- (above diagonal) and genetic correlations (below diagonal) between all traits (standard errors in brackets).

Trait*	BW	L	HL	W	CL	HW	FW	H%	F%
BW		0.89 (0.01)	0.80 §	0.90 (0.01)	0.84 §	0.81 §	0.95 (0.00)	-0.44 (0.02)	0.48 (0.02)
L	0.87 (0.08)		0.86 §	0.80 (0.01)	0.97 §	0.77 (0.01)	0.82 (0.01)	-0.38 (0.03)	0.46 (0.02)
HL	0.76 (0.09)	0.94 (0.03)		0.72 §	0.71 §	0.74 §	0.72 §	-0.27 §	0.38 (0.03)
W	0.92 (0.05)	0.77 (0.16)	0.55 (0.14)		0.76 (0.02)	0.70 (0.02)	0.86 (0.01)	-0.45 §	0.51 §
CL	0.87 (0.06)	0.98 §	0.82 (0.08)	0.84 (0.17)		0.71 (0.01)	0.79 (0.01)	-0.39 (0.02)	0.45 (0.02)
HW	0.99 §	0.98 (0.07)	0.96 (0.03)	0.75 (0.17)	0.92 (0.12)		0.73 (0.01)	0.10 (0.03)	0.32 (0.03)
FW	0.99 (0.01)	0.89 (0.10)	0.70 (0.11)	0.94 (0.06)	0.91 (0.11)	0.97 (0.10)		-0.45 (0.02)	0.71 (0.01)
H%	-0.78 (0.21)	-0.67 (0.30)	-0.36 §	-0.98 §	-0.75 (0.28)	-0.64 (0.45)	-0.81 (0.19)		-0.36 (0.02)
F%	0.74 (0.18)	0.62 (0.24)	0.47 (0.29)	0.98 §	0.60 (0.26)	0.62 (0.30)	0.81 (0.13)	-0.94 (0.21)	

* for legend see Table 1

§ Variance parameters were fit at the boundary of the parameter space; consequently ASREML does not provide standard errors

Genetic effects of founder strains are presented in Table 4. For body weight, the genetic effect of the GIFT strain was highest (98.5g), whereas the genetic effect of the GÖTT strain was lowest (-128.4g). The difference between these strains was 226.9g. In general, the highest genetic values for body measurements where shown in the GIFT strain and the lowest in the GÖTT strain with the exception of head yield. Average genetic values for fillet yield were highest in the GIFT strain (1.94%) followed by the IDRC- (0.23%), AIT- (-0.38%) and GÖTT

Table 4. Genetic effects of four founder strains (AIT, GIFT, IDRC and GÖTT) for all traits expressed as a deviation from zero.

Trait*	AIT	GIFT	IDRC	GÖTT
BW (g)	32.5 (44.21)	98.5 (29.66)	-2.6 (43.59)	-128.4 (37.32)
L (cm)	0.63 (0.47)	0.83 (0.32)	-0.08 (0.47)	-1.38 (0.40)
HL (cm)	0.25 (0.15)	0.30 (0.10)	-0.01 (0.15)	-0.54 (0.13)
W (cm)	0.07 (0.57)	0.33 (0.58)	0.03 (0.58)	-0.44 (0.58)
CL (cm)	0.45 (0.34)	0.45 (0.23)	-0.05 (0.33)	-0.85 (0.29)
HW (g)	5.73 (9.02)	12.43 (6.02)	1.93 (8.79)	-20.08 (7.60)
FW (g)	10.18 (21.66)	53.38 (14.52)	-4.23 (21.31)	-59.33 (18.27)
H% (%)	-0.17 (4.24)	-1.49 (4.39)	-0.09 (4.30)	1.74 (4.34)
F% (%)	-0.38 (5.29)	1.94 (5.48)	0.23 (5.37)	-1.78 (5.41)

* for legend see Table 1

(-1.78%) strains, the difference between the best and worst was 3.7%. For fillet weight, the absolute difference between the GIFT- and GÖTT strains was 112.7 grams (53.38g+59.33g).

Accuracies of selection indexes are presented in Table 5, where the breeding goal consisted of fillet weight. To place these values in perspective, the accuracy of hypothetical direct selection for fillet weight was included: 0.46. The accuracy of the single trait indexes was at its maximum (0.47) when body weight was used. As soon as body weight was excluded from the index, the accuracy of single trait indexes dropped to values around 0.42. Differences between the use of length (0.41) or width (0.43) were small with an advantage for width. When length or width was used in combination with body weight, the accuracy of the index with body weight and width was marginally higher.

Table 5. Accuracies of selection (r_{IH}) for different indexes evaluated for a breeding goal containing fillet weight as target trait ($H=1 \cdot FW$)*.

Index	r_{IH}
$I=b_1 \cdot FW$ *(hypothetical direct selection)*	0.46
$I=b_1 \cdot BW$	0.47
$I=b_1 \cdot L$	0.41
$I=b_1 \cdot W$	0.43
$I=b_1 \cdot BW+b_2 \cdot L$	0.47
$I=b_1 \cdot BW+b_2 \cdot W$	0.47
$I=b_1 \cdot L+b_2 \cdot W$	0.45
$I=b_1 \cdot BW+b_2 \cdot L+b_3 \cdot W$	0.47

* Equilibrium genetic parameters were used, calculated using the computer program SelAction (Rutten et al., 2002).

Discussion

Methods

In this study genetic parameters were estimated for fillet traits and related body measurements. The most important parameters, heritabilities for body weight (BW: 0.26) and fillet weight (FW: 0.24) were moderate and for fillet yield low (F%: 0.12). Due to large additive genetic variance, prospects for selection on body weight and fillet weight are excellent. Response in both traits can be achieved, either by direct- (in the case of body weight) or indirect selection. In the case of indirect selection for fillet weight, body measurements can be used since heritabilities were moderate and genetic correlations between body measurements and fillet weight were all high with the exception of head length (HL). In the case of fillet yield, both the phenotypic variance and the heritability were low.

Standard errors of heritabilities and c^2 were relatively high, but heritabilities of body weight, fillet weight, fillet yield and head weight (HW) were significantly different from zero. The family effect, although never significant, was retained in the model because omitting this effect can inflate additive genetic variances (Martinez et al., 1999; Pante et al., 2002). The slaughter data used to in this study was collected on a selected population. Therefore, a bivariate setting

was used for estimation of parameters, where one of the two traits was collected on all animals. In this way unbiased variance estimates for the traits with missing values were obtained (Ouweltjes et al., 1988). In the current study, data from the non-selected animals was available, which is an uncommon situation. Mostly, data on only the selected animals is available. Hence, the number of animals with observations was relatively large and therefore no deviations from the unselected population's variance were expected when performing univariate instead of bivariate analyses. Additional analyses confirmed this.

The population used in this study originated from four genetically different strains (Rutten et al., 2004b). All combinations off crosses between strains were available as well as pure strain individuals. However, in the model no specific measures were taken to correct for possible effects of heterosis although these have been found in Nile tilapia before (Bentsen et al., 1998). We assumed that possible effects of heterosis would be absorbed within the effect of family and therefore did not influence the estimated genetic parameters. The presence of maternal half-sib families in the family structure can be regarded as a potential source of bias in the genetic parameters. Because of insufficient depth in the pedigree, we were not able to correct for possible maternal (genetic) effects. In full-sib families, possible variance due to maternal genetic effects could be absorbed by the random family effect. In maternal half-sib families, however, variance due to maternal effects could be absorbed by the additive genetic component. Depending on the magnitude of the maternal effects, the presented heritabilities might be biased upwards. However, in a cross of *Oreochromis mossambicus* and *O. hornorum*, Gall and Bakar (2002) showed that the maternal genetic variance as a proportion of the phenotypic variance was low (0.02) and therefore the bias in this study is expected to be small.

Literature

Comparing heritabilities and correlations found in this study to literature values is difficult, since they are lacking for most traits. Velasco et al. (1995) reported heritabilities based on the sire component of variance based on male- and female offspring separately and for a number of traits: body weight (h^2_s males=0.61, h^2_s females=0.24), length (h^2_s males=0.48, h^2_s females=0.44), head weight (h^2_s males=0.17, h^2_s females=0.48) and fillet weight (h^2_s males=0.63, h^2_s females=0.30). Taking the average of the heritability for males and females, we

concluded that the corresponding heritabilities found in our study are lower. The age at which Velasco et al. (1995) measured body weight was not reported. Furthermore, the fish were kept in ponds which could have had an effect on the estimated variances. Whether the absence of a family effect in the model of Velasco et al. (1995) could have inflated variance estimates (Martinez et al., 1999; Pante et al., 2002) was unclear.

Fillet yield found in the current study was higher by 1.6% (37.3% versus 35.7%) compared to our previous study (Rutten et al., 2004a). However, both studies differed on a number of points: circumstances under which fry were produced and reared, the (genetic) contribution of founder strain material to the population of fish and the filleting method. In the previous study, fish were filleted by hand whereas they were filleted by machine in the current study. Filleting by hand is very precise, although filleting Nile tilapia in particular requires a lot of strength and fatigue will have its effect on the filleting precision after some time. Filleting machines are tuned to achieve the highest precision within a certain range of fish weights which is mostly much smaller than the range of fish weights that can be found within a population. Outside of this range the filleting precision will be lower. The filleting method was probably the most likely cause for the increased fillet yield because visual inspection of the automatically cut fillets suggested that a bigger proportion of the belly flap remained attached to the fillets.

Comparison of the phenotypic correlations found in this study and in the study by Rutten et al. (2004a) revealed some differences. The absolute differences in phenotypic correlations among body weight, fillet weight and body measurements in both studies were 0.08 on average. However, the absolute differences in phenotypic correlations among fillet yield and the other traits in both studies were 0.34 on average and were always higher in the current study. The reason for this remains unclear.

Prospects for selection on fillet weight

In aquaculture, interest in fillet yield has always prevailed. However, in production for the European market, breeding only to improve fillet yield seems irrelevant because a farms total output is of primary interest. Small fish with high fillet yield are certainly not preferred. In this study we showed that genetic variation for fillet yield is relatively small and prospects for breeding are not good. Therefore, breeding goals for fish species should always include a

measure of weight, and this can be either body weight or fillet weight. Fillet weight is directly related to a producer's income, and is the most important trait to our opinion, and fillet yield is less relevant. When a breeding program targets improvement of fillet weight, the fish's genetic makeup will determine whether this is realized by improving fillet yield at equal body weight, or improving body weight at equal fillet yield, or as a combination of both. Given the genetic correlations found in this study, the development of fillet weight is probably mostly realized by improvement of body weight at equal fillet yield. Economically, an essential addition to the breeding goal would be a trait directly related to production costs e.g. nutrient efficiency. Determining efficiency of nutrient use however, is a hard task in aquaculture (Gjedrem, 1983; Kinghorn, 1983).

One of the aims of this paper was to clarify whether mass selection can result in improved fillet traits. In our calculations the breeding goal consisted of fillet weight only. The inclusion of body weight in the index always increases the selection response that can be expected compared to the use of only body measurements. In fact, by using body weight as index trait, roughly the same selection response can be obtained than hypothetical direct selection for fillet weight would. The fact that the use of body weight resulted in a higher accuracy than the use of fillet weight is probably due to a combination of numerical optimization within the program SelAction, and high standard errors on the estimated variance components. The highest accuracy of selection (0.47) was achieved when body measurements were included in the index additionally to body weight, but the differences were small. When only width is used in the index, the selection response in fillet weight would be (0.43-0.47)/0.47=8.5% lower than when using only body weight in the index. We restricted the index traits to body weight, length and width, because these are easily measurable. Nowadays, automated systems are available to classify animal based on body characteristics, such as mechanical sorting machines, image processing and even sorting based on laser techniques. When these methods can be implemented in a breeding program, large quantities of fish could be evaluated and very high selection intensities could be achieved. When the extra gain from using e.g. sorting machines and high selection intensities compensates for the loss of accuracy by using only body measurements, these can become interesting as index traits.

In conclusion, the use of body measurements does not seem to have a large added value for mass selection on fillet weight, especially when equal efforts are required for measuring body weight or body measurements of a fish. Using body weight as index trait for indirect selection on fillet weight is very efficient. However, when large quantities of fish have to be measured, the use of sorting machines and selection on width is a good alternative. In that case, an 8.5% reduction of expected genetic response is achieved compared to the use of body weight as index trait. For now, however, body weight is still the best predictor to improve fillet weight.

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References

Bentsen, H.B., Eknath, A.E., Palada-de Vera, M.S., Danting, J.C., Bolivar, H.L., Reyes, R.A., Dionisio, E.E., Longalong, F.M., Circa, A.V., Tayamen, M.M., Gjerde, B., 1998. Genetic improvement of farmed tilapias: growth performance in a complete diallel cross experiment with eight strains of Oreochromis niloticus. Aquaculture 160, 145-173.

Bosworth, B.G., Libey, G.S., Notter, D.R., 1998. Relationships among total weight, body shape, visceral components, and fillet traits in Palmetto Bass (Stripped Bass female *Morone saxatilis* x White Bass male *M. chrysops*) and Paradise Bass (Stripped Bass female M. saxatilis x Yellow Bass male M. mississippiensis). J. World Aquaculture Soc. 29, 40-50.

Bosworth, B.G., Holland, M., Brazil, B.L., 2001. Evaluation of ultrasound imagery and body shape to predict carcass and fillet yield in farm-raised catfish. J. Anim. Sci. 79, 1483-1490.

Cibert, C., Fermon, Y., Vallod, D., Meunier, F.J., 1999. Morphological screening of carp *Cyprinus carpio*: relationship between morphology and fillet yield. Aquat. Liv. Res. 12, 1-10.

Gilmour, A.R., B J Gogel, B R Cullis, S J Welham and R Thompson, 2002. ASReml User Guide Release 1.0. VSN International Ltd, 5 The Waterhouse, Waterhouse Street, Hemel Hempstead, HP1 1ES, UK

Gjedrem, T., 1983. Genetic variation in quantitative traits and selective breeding in fish and shellfish. Aquaculture 33, 51-72.

Kinghorn, B.P., 1983. A review of quantitative genetics in fish breeding. Aquaculture 31, 283-304.

Martinez, V., Neira, R., Gall, G.A.E., 1999. Estimation of genetic parameters from pedigreed populations: lessons from analysis of alevin weight in Coho salmon (*Oncorhynchus kisutch*). Aquaculture 180, 223-236.

Ouweltjes, W., Schaeffer, L.R., Kennedy, B.W., 1988. Sensitivity of methods of variance component estimation to culling type of selection. J. Dairy Sci. 71, 773-779.

Pante, M.J.R., Gjerde, B., McMillan, I., Misztal, I., 2002. Estimation of additive and dominance genetic variances for body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 204, 383-392.

Quaas, R.L., 1988. Additive genetic model with groups and relationships. J. Dairy Sci. 71, 1338-1345.

Rutten, M.J.M., Bijma, P., Woolliams, J.A., Arendonk, J.A.M. van, 2002. SelAction: Software to predict selection response and rate of inbreeding in livestock breeding programs. J. Hered. 93, 456-458.

Rutten, M.J.M., Bovenhuis, H., Komen, H., 2004a. Modeling fillet traits based on body measurements in three Nile tilapia strains (*Oreochromis niloticus* L.). Aquaculture 231, 113-122.

Rutten, M.J.M., Komen, H., Deerenberg, R.M., Siwek, M., Bovenhuis, H., 2004b. Genetic characterization of four strains of Nile tilapia (*Oreochromis niloticus* L.) using microsatellite markers. Animal Genetics 35, 93-97.

Rutten, M.J.M., Komen, H., Bovenhuis, H., 2005. Longitudinal genetic analysis of Nile tilapia (*Oreochromis niloticus* L.) body weight using a random regression model. Aquaculture, in press.

Velasco, R.R., Janagap, C.C., De Vera, M.P., Afan, L.B., Reyes, R.A., Eknath, A.E., 1995. Genetic improvement of farmed tilapias: estimation of heritability of body and carcass traits of nile tilapia (*Oreochromis niloticus*). Aquaculture 137, 280-281.

Westell, R.A., Quaas, R.L., Van Vleck, L.D., 1988. Genetic groups in an animal model. J. Dairy Sci. 71, 1310-1318.

Chapter V

Longitudinal genetic analysis of Nile tilapia (*Oreochromis niloticus* L.) body weight using a random regression model

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ABSTRACT

Genetic parameters for body weight at ages over approximately 120 days are scarce in Nile tilapia. In this study, genetic parameters for body weight in Nile tilapia were estimated for ages ranging from 100 days to 326 days. To this end, five repeated observations of body weight were collected on 2483 pedigreed fish. The fish originated from a population descending from four different genetic backgrounds. Genetic parameters were estimated using a random regression model with covariance functions. The heritability of body weight was fairly constant around 0.2, which offers good prospects for selection on body weight. Genetic correlations were estimated between all ages (100-326 days). At higher ages, genetic correlations were clearly more stable: for example genetic correlations were over 0.9 between the age of 100 days and all ages up to 115 days of age; at higher ages genetic correlations were over 0.9 between the age of 223 days and all ages up to 326 days of age. The estimated genetic parameters showed that early selection results in higher selection response than direct selection, when the target trait is body weight at the age of 326 days. This is due to somewhat higher heritabilities at lower age and a shortened generation interval. Furthermore, evidence was found for genetic differences in growth patterns of fish of different strains. This means that the possibility to change the shape of body weight curves by selection exists and that the choice of strains should depend on the target market weight of the production chain. From the raw data we concluded that differences in body weight between male- and female fish were significant already at early ages (100 days). Results from a bivariate genetic analysis, where body weight of male- and female fish are treated as separate traits, suggest that body weight in male and female fish is most likely controlled by the same genes. Prospects to decrease the difference between mature male- and female body weight by selection are therefore unfavorable in Nile tilapia.

Introduction

Interest in genetic improvement of Nile tilapia is increasing rapidly. In the Netherlands, a new synthetic population was created based on four strains of Nile tilapia previously described (Rutten et al., 2004b). This population is kept in a closed recirculation system and is subjected to a breeding program for mature body weight. To facilitate the design and evaluation of breeding programs, genetic parameters are required. When genetic parameters are available, not only the amount of response from selection can be predicted, but also the effectiveness of alternative information sources and the increase of a populations inbreeding coefficient due to

a systematic breeding program can be evaluated (Bijma et al., 2000; Rutten et al, 2002). Genetic parameters for body weight of this population are lacking, however, and have to be estimated to serve this purpose.

Several studies have reported genetic parameters (mostly heritabilities) for body weight in Nile tilapia in the past (Tave and Smitherman, 1980; Lester et al., 1988; Teichert-Coddington and Smitherman, 1988; Kronert et al., 1989; Oldorf et al., 1989; Brzeski and Doyle, 1995; Eknath et al., 1998; Bolivar and Newkirk, 2002; Gall and Bakar, 2002). These parameters were estimated for body weight at ages less than 200 days and often in small data sets. Tilapia production for the European market, however, targets body weights over 600 grams, for which at least 300 days are needed. To our knowledge, genetic parameters (heritabilities) for the age of 360 days have been published only by Langholz (1987). Genetic correlations for body weight at different ages have not been published before in Nile tilapia whereas these can provide valuable information for possibilities of early selection.

The aim of this study was, therefore, to estimate genetic parameters of body weight of Nile tilapia up to the age of at least 300 days. For this purpose, a random regression model was used which allows estimation of genetic variances for all ages, and genetic covariances between all ages present in the data.

Materials and methods

Experiment

Parents originating from four different strains of Nile tilapia (AIT, GIFT, IDRC and Göttingen; Rutten et al., 2004a, 2004b) were randomly mated to produce progeny in 73 full-sib families. All matings were performed in vitro, after hand-stripping females for eggs and males for sperm. Transport of fish from the production- to the breeding facilities probably caused fertility problems of female fish. Since parents originated from 4 different strains, an attempt was made to maximize the contribution of parents to the next generation. Therefore, each female was crossed with 2 males. Eventually, 40 dams and 56 sires contributed and paternal- as well as maternal half-sib families were present.

After incubation of eggs, 150 fry of each full-sib family were raised in separate tanks of approximately 25 liters, all attached to the same recirculation system. Due to the fact that spawning of females was not synchronized, all families were produced over a period of 12 weeks. When the fry reached an average weight of approximately 5 grams, 100 fry per family

were individually tagged using Floy® fingerling tags. First, 17 families were tagged and then combined into one tank (4500 l). One week later the next 18 families were tagged and combined together with the first 17 families. These 35 families will be referred to as group one (GR1). At the moment of combining, 1500 untagged fish of randomly chosen from the 73 families were added to the tank, to increase the stocking density to commercial levels (3500+1500=5000 fish in total). For families 36 to 73 the scenario was similar, except that they were tagged in three batches. This group will be referred to as GR2. In total 24 dams and 35 sires contributed to GR1 and 27 dams and 34 sires contributed to GR2. Of these parents, 11 dams and 13 sires contributed to both GR1 and GR2, so genetic links between groups GR1 and GR2 were available. In this way confounding of effects of environment and group were avoided. A timeline of the experiment representing all activities with respect to combining and splitting of tanks and measuring of the fish is presented in Figure 1.

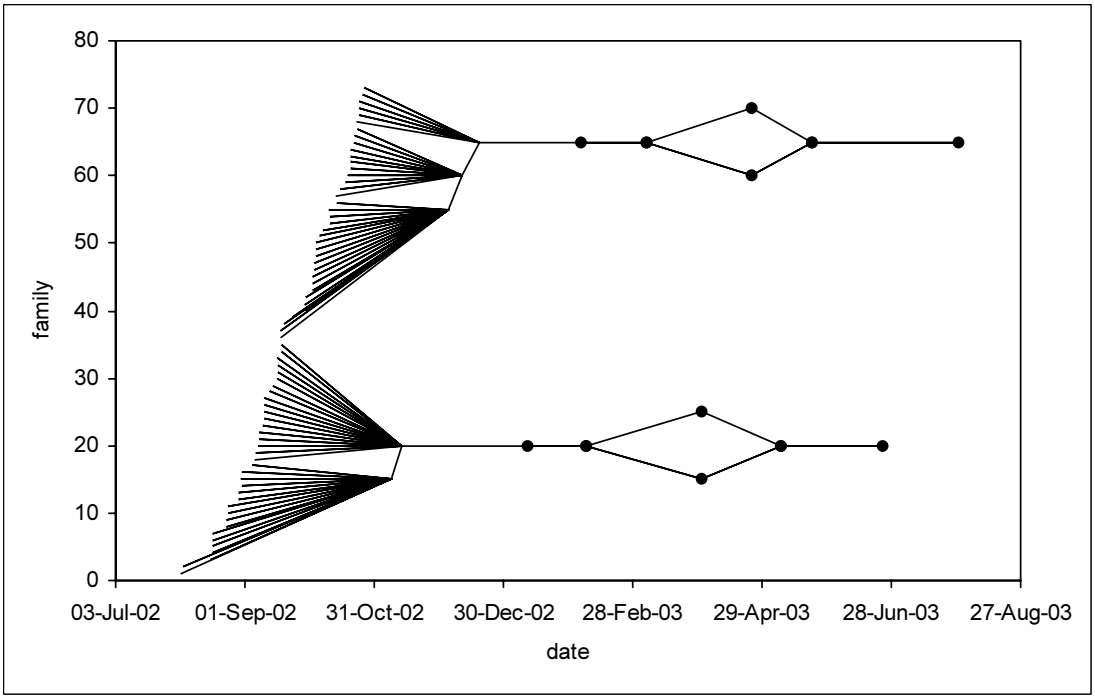

Figure 1. Timeline of the experiment: Each separate line (from the left to the right) indicates one of 73 separate families being reared in a separate tank for a period of time. Five measurement-dates are indicated with dots (●). After measurement-date 2, groups were split, and combined again after measurement 4. Where lines come together families were grouped and eventually, two groups were maintained (lower=group 1 (GR1); upper=group 2 (GR2)).

During the rearing period which followed, five measurements of weight per fish were taken at five different moments for each of the two groups (Figure 1). After the second measurement,

GR1 and GR2 were split into two groups, in such a way that 50 fish of a family went into one tank, and the other 50 fish into the other tank (4500 l). After the fourth measurement, four groups were brought back to two groups according to the original arrangement, and transferred into two tanks (20000 l) where they remained until the end of the experiment.

When measurements of weight were taken, all fish were netted and anaesthetized in batches using C-Date 222 (tricaine methanesulfonate / MS222). Subsequently, weight and tag-number were recorded. After measurement number two, the number of fish carrying tags decreased, therefore Floy® tags were replaced with PIT tags at measurement number three. At measurement number three, sex of the fish was determined. In total 18560 measurements of body weight of 5927 pedigreed animals were recorded. Animals with less than five measurements were discarded from the data set to overcome convergence problems. Eventually, 12430 records of 2486 animals were available for analysis.

Univariate genetic analysis

Univariate genetic analyses were performed to estimate variance components for each of five measurements separately. The data was corrected for fixed effects of sex and tank and a covariate for the age of the animal was included (all ages in this paper refer to age post-hatching). Additive genetic- and (full-sib) family random effects were included in the model. The family effect can include maternal (genetic), non-additive genetic and common environmental effects. Genetic groups were included in the model to correct for different genetic means of founder strains (Westell et al., 1988; Quaas, 1988). In matrix notation the model can be written as

$$y=Xb+Z_1Qg+Z_2u^*+Z_3f+\varepsilon$$

with expectations

$$E(y)=Xb \text{ and } E(u^*)=E(f)=E(\varepsilon)=0$$

and variance

$$V(y)=Z_2AZ_2'\sigma^2_{u^*}+Z_3IZ_3'\sigma^2_f+I\sigma^2_e$$

where, X is an incidence matrix for the fixed effects vector b (including a covariate for age), g is a vector with genetic group means, u^*, f and ε are vectors with additive genetic, family and residual random effects, Z_1, Z_2 and Z_3 are incidence matrices, Q is an incidence matrix relating base animals to their genetic group means, A is the additive genetic relationship matrix and I

are identity matrices. Estimated animal additive genetic effects can be written as $\hat{u}=Q\hat{g}+\hat{u}^*$ (Quaas, 1988).

Using the same model terms, an additional bivariate genetic analysis was performed, where body weight of male and female fish were treated as different traits. Additive genetic covariances between male and female body weight were estimated.

Random regression genetic analysis

A random regression model using a covariance function (Meyer and Hill, 1997; Meyer, 1998) was used to do a second analysis of the data. Orthogonal polynomial functions of order two (i.e. with three regression coefficients) were used to describe both fixed and random effects. Fixed curves for the effect of sex and tank were included. In the random part of the model, curves for the additive genetic effect, permanent environmental effect, (full-sib) family effect and error effect to the random regressions of each consecutive age were included. Permanent environmental effects take into account the residual covariance between repeated measurements (Apiolaza and Garrick, 2001). In addition to the age depending error terms, one overall error effect was modeled. Note, that the model allows for heterogeneous error variances in this way (Nobre et al., 2003). Genetic groups were included in the model and in matrix notation the model can be written as

$$y=Xb+ZQg+\Phi_{u*}k_{u*}+\Phi_{pe}k_{pe}+\Phi_{f}k_{f}+\Phi_{e}k_{e}+\varepsilon$$

with expectations

$$E(y)=Xb \text{ and } E(\Phi_{u*}k_{u*})=E(\Phi_{pe}k_{pe})=E(\Phi_{f}k_{f})=E(\Phi_{e}k_{e})=E(\varepsilon)=0$$

and variance

$$V(y)=\Phi_{u*}K_{u*}\Phi_{u*}'+\Phi_{pe}K_{pe}\Phi_{pe}'+\Phi_{f}K_{f}\Phi_{f}'+\Phi_{e}K_{e}\Phi_{e}'+I\sigma^2_{\varepsilon}$$

(note that $V(R)=\Phi_{e}K_{e}\Phi_{e}'+I\sigma^2_{\varepsilon}$)

where, y is a vector of weights of individual fish, X is an incidence matrix for the vector of fixed effects b, Z is an incidence matrix for the vector of genetic group effects g, Q is an incidence matrix relating base animals to their genetic group means (curves are fitted for each level in Xb and ZQg), Φ_{u*}, Φ_{pe}, Φ_{f} *and* Φ_{e} are matrices with orthogonal polynomial coefficients corresponding to matrices with additive genetic, permanent environmental, family and error random regression coefficients k_{u*}, k_{pe}, k_{f} and k_{e} respectively and ε is a vector of overall error terms. Furthermore, K_{u*}, K_{pe}, and K_{f} are variance-covariance matrices (of order equal to the order of the polynomial used) of additive genetic, permanent environmental and family random

regression coefficients, K_e is a diagonal matrix containing variances of error random regression coefficients guaranteeing independence within random regression error terms, I is an identity matrix of order equal to the number of observations indicating constant residual variance σ^2_ε and R is the total heterogeneous residual variance matrix.

Solutions to the model were generated using restricted maximum likelihood (REML) implemented in ASReml software (version 1.10, 15 oct 2003; Gilmour et al., 2002).

Additive genetic, permanent environmental, family and error variances were calculated for each age using a covariance function. For the additive genetic variance at e.g. age 200: $var(u_{a=200})=cov(u_{a=200}, u_{a=200})=\varphi_{200}K_{u*}\varphi_{200}'$, where φ_{200} is the row of Φ_{u*} corresponding to the age of 200 days and K_u is the additive genetic (co)variance matrix. Subsequently, heritabilities were calculated at each age, as the ratio of additive genetic- to phenotypic variance (h^2). The ratios family variance to phenotypic variance and permanent environmental variance to phenotypic variance are denoted as c^2 and pe^2. Genetic correlations for body weight at different ages were calculated in a similar way.

Results

Descriptive statistics

In Figure 2, the average phenotypic body weight curves, based on raw data, are plotted for male and female fish. It shows that fish were around 50 grams at the age of 122 days (measurement 1). Towards measurement 2 at the age of 151 days, body weight increased rapidly, for male fish the increase was higher. After measurement 2, the increase of body weight showed a linear pattern towards 744 grams for male fish and 419 grams for female fish at measurement 5 and the age of 293 days (see also: Table 1). Variation increased rapidly with age (Figure 2). All body weight distributions were tested normal using Kolmogorov-Smirnov tests of normality (P<0.01). Student's t-tests indicated that the difference in mean body weight of male and female fish was significant at all measurements (P<0.001). At measurement 5, body weights of the largest female fish were approximately equal to the average body weight of male fish. Body weight curves did not bend towards a plateau near measurement 5.

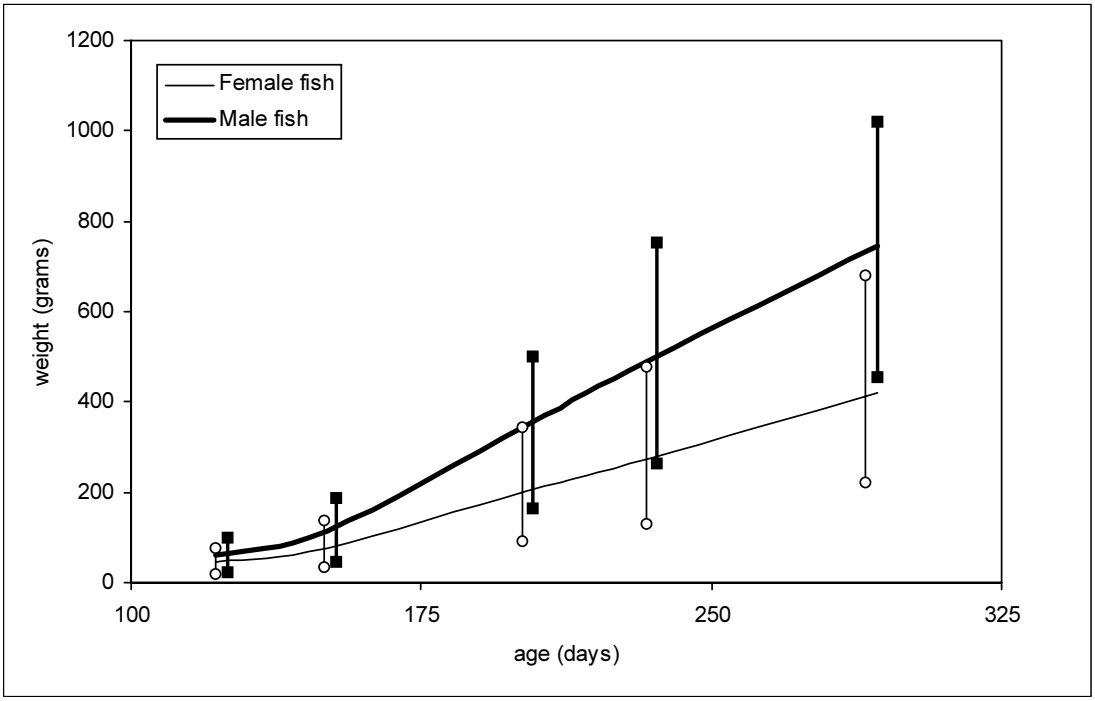

Figure 2. Phenotypic body weight curves for male- and female fish, and ranges containing observations from the 5th to the 95th percentile.

Table 1. Average age (days), mean live body weights (μ in grams) and standard deviation of live body weight (σ in grams) for all-, male- and female- fish at each measurement (1-5).

		Measurement				
		1	2	3	4	5
Average age		122.1	151.0	202.2	234.2	292.9
All fish n=2483	μ	55.5	100.3	286.1	403.6	609.8
	σ	22.3	42.9	114.9	170.6	226.4
Male fish n=1459	μ*	61.9	116.3	346.2	492.5	744.0
	σ	23.2	42.5	96.6	146.8	172.4
Female fish n=1024	μ*	46.4	77.6	200.9	276.8	418.7
	σ	17.4	31.8	78.8	110.0	140.5

* t-tests indicated that mean body weights of male- and female fish were significantly different at each measurement (P<0.001).

Univariate and bivariate genetic analyses

Results of the univariate and bivariate genetic analyses are shown in Table 2. The heritability of body weight for all fish varied from 0.16 at measurement 1 to 0.26 at measurement 5. Standard errors were relatively high, so that these heritabilities were neither significantly different from zero nor different from each other (α=0.05). The ratio of family variance to phenotypic variance (c^2) for all fish decreased from a value of 0.21 at measurement 1 to a value of 0.09 at measurement 5. Standard errors for c^2 were smaller and c^2 differed significantly from zero at measurements 1 and 2. Note, that the phenotypic variance at measurement 5 was almost 60 times larger than at measurement 1, whereas the additive genetic variance was almost 100 times larger.

Results from the bivariate analysis indicated that the average heritability for male and female fish was lower compared to the univariate estimate but higher at all other measurements, especially at measurement 5. The average c^2 for male and female fish showed slightly higher values compared to the analysis in univariate setting. Heritabilities of female body weight were significantly different from zero at measurements 3, 4 and 5. The ratio common environmental variance to phenotypic variance (c^2) was significant for both male- and female fish at measurements 1 and 2. At measurement 3, c^2 for only male fish was significant. Genetic correlations were generally high and not significantly different from unity.

Random regression genetic analysis

Variance components estimated with the random regression model are shown in Figure 3. Permanent environmental variance was high and developed in a quadratic manner as age increased. Additive genetic variance, however, was lower, and developed in an almost linear manner. Both error- and family variance were relatively low, but the error variance fluctuated in time between approximately 1000 and 5000 g^2. The modeled phenotypic variance, as an aggregate of the former four components, showed an initial decrease but increased in a quadratic manner from the age of approximately 125 days.

Table 2. Additive genetic- (σ^2_u), family- (σ^2_f), error- (σ^2_ε) and phenotypic variance (σ^2_p), heritability (h^2) and the ratio of common environmental to phenotypic variance (c^2) with standard errors between brackets for all-, male- or female fish and the genetic correlation between male and female body weight (r_g) with standard errors between brackets for measurements (M) 1 to5.

M	Data	σ^2_u	σ^2_f	σ^2_ε	σ^2_p	h^2	c^2	r_g
	All	62	83	246	391	0.16(0.15)	0.21(0.07)	
1§	Male	52	74	299	425	0.12(0.13)	0.17(0.06)	
	Female	23	98	166	287	0.08(0.21)	0.34(0.11)	0.99*
	All	329	199	865	1393	0.24(0.16)	0.14(0.07)	
2	Male	335	276	1250	1861	0.20*	0.17(0.04)	
	Female	288	133	503	924	0.31*	0.14(0.05)	0.99*
	All	1481	802	4814	7097	0.21(0.13)	0.11(0.06)	
3	Male	1059	1252	5976	8287	0.13(0.14)	0.15(0.07)	
	Female	1794	596	2597	4987	0.36(0.18)	0.12(0.08)	0.99(0.38)
	All	2778	1405	9377	13560	0.20(0.13)	0.10(0.06)	
4	Male	3081	1690	10945	15716	0.20(0.14)	0.11(0.06)	
	Female	4056	699	4821	9576	0.42(0.16)	0.07(0.06)	0.85(0.25)
	All	6033	2080	15119	23231	0.26(0.14)	0.09(0.06)	
5	Male	7463	2759	17988	28210	0.26(0.14)	0.10(0.06)	
	Female	9968	396	6277	16641	0.60(0.18)	0.02(0.06)	0.92(0.19)

§ Due to convergence problems in bivariate setting, variance parameters were estimated in univariate setting. Subsequently, univariate parameters were fixed in a bivariate analysis to estimate the additive genetic covariance.

* Values were fixed at the boundary of the parameter space and consequently no standard errors were available.

The heritability, c^2 and pe^2 are shown in Figure 4. The heritability increased rapidly from 0.08 at low age to approximately 0.20 at the age of 140 days and increased slowly to 0.24 at the age of 250 days. After that, the heritability decreased to 0.15. Family variance (c^2) varied marginally, but was always around 0.05. Permanent environmental variance (pe^2) increased very rapidly towards an age of 150 days and from then onwards, kept increasing more steadily towards higher ages.

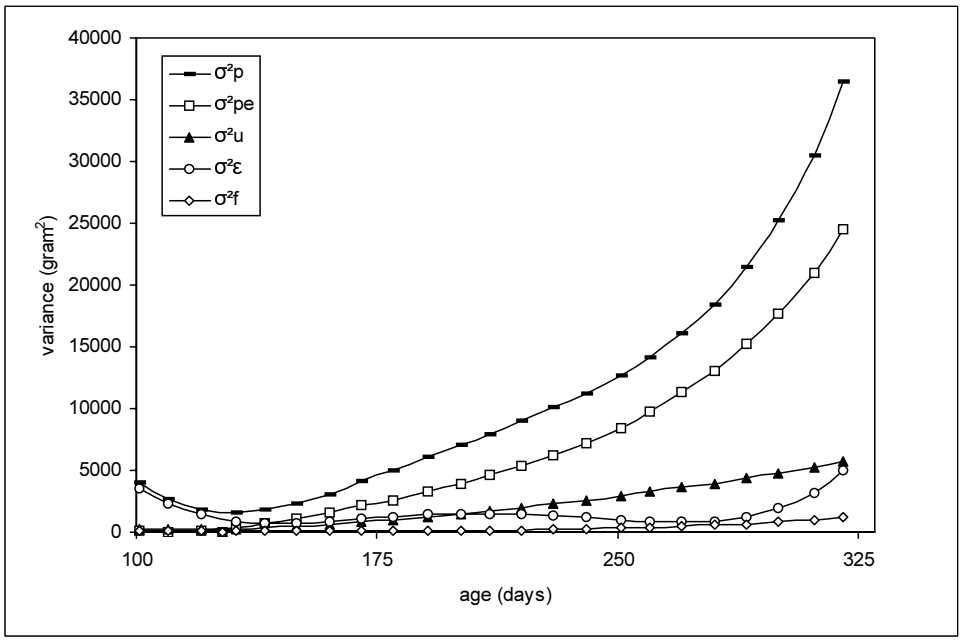

Figure 3. Continuous phenotypic- (σ^2_p), additive genetic- (σ^2_u), permanent environmental- (σ^2_{pe}), family- (σ^2_f) and heterogeneous error variance (σ^2_ε) from the age of 101 days to the age of 326 days, estimated with a random regression animal model.

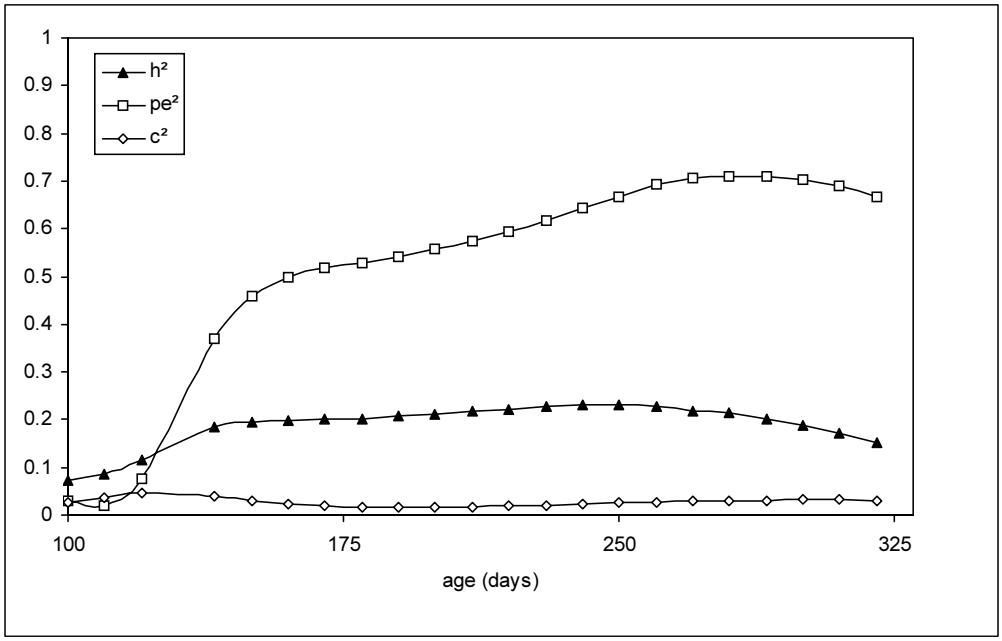

Figure 4. Additive genetic- (h^2), family- (c^2) and permanent environmental variance (pe^2) as a proportion of the phenotypic variance from the age of 101 days to the age of 326 days, estimated with a random regression model.

A contour-plot of the genetic correlations is shown in Figure 5. All genetic correlations in the area which includes the diagonal were over 0.9. When evaluating the genetic correlation between the age of 326 days and lower ages in Figure 5 (i.e. going down from upper right to lower right) the genetic correlation remained over 0.8 until the age of approximately 180 days is passed. After that, the genetic correlation dropped more rapidly. However, evaluating the genetic correlation between the age of 100 days and higher ages (i.e. going up from lower left to upper left) the genetic correlation remained over 0.8 only until the age of approximately 135 days is passed.

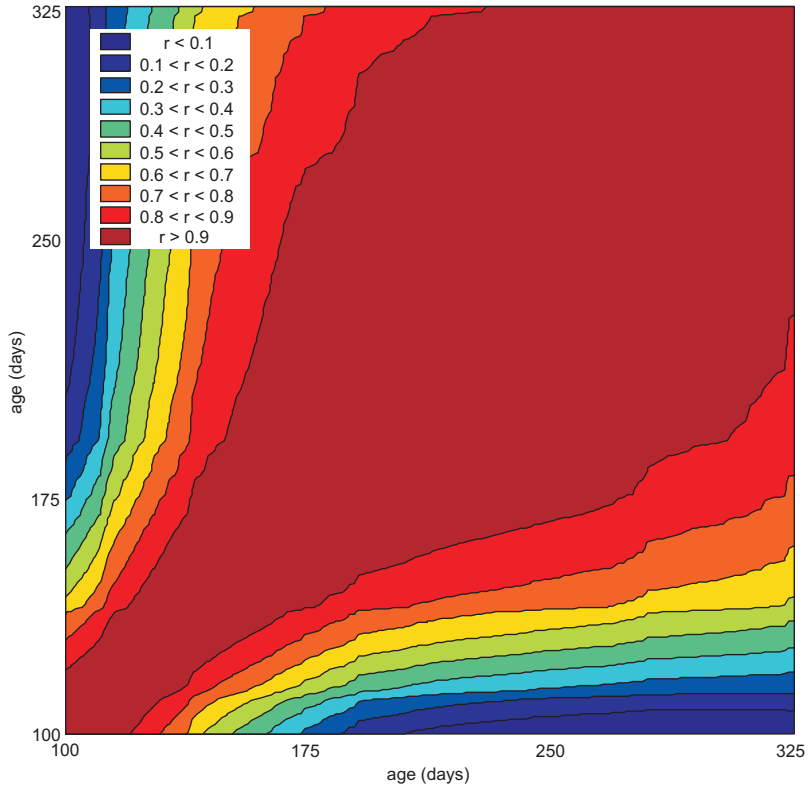

Figure 5. Genetic correlations between body weights of all ages between 101 and 326 days, estimated with a random regression model. Genetic correlations on the diagonal (from lower left to upper right) were equal to 1, all genetic correlations away from the diagonal were smaller than 1.

Average breeding values of strains are shown in Figure 6. Large genetic differences between growth patterns of strains were found. At the age of 326 days, the maximum difference was almost 280 grams (AIT versus GÖTT). Differences between the AIT, GIFT and IDRC strains were relatively small. Interestingly, differences between the strains average breeding values for

body weight varied greatly at different ages, both in magnitude and in rank. At the age of 175 days for example, the IDRC strain showed a slightly higher breeding value for body weight than the GIFT strain but at the age of 325 days, not only the rank had changed but the difference was bigger as well.

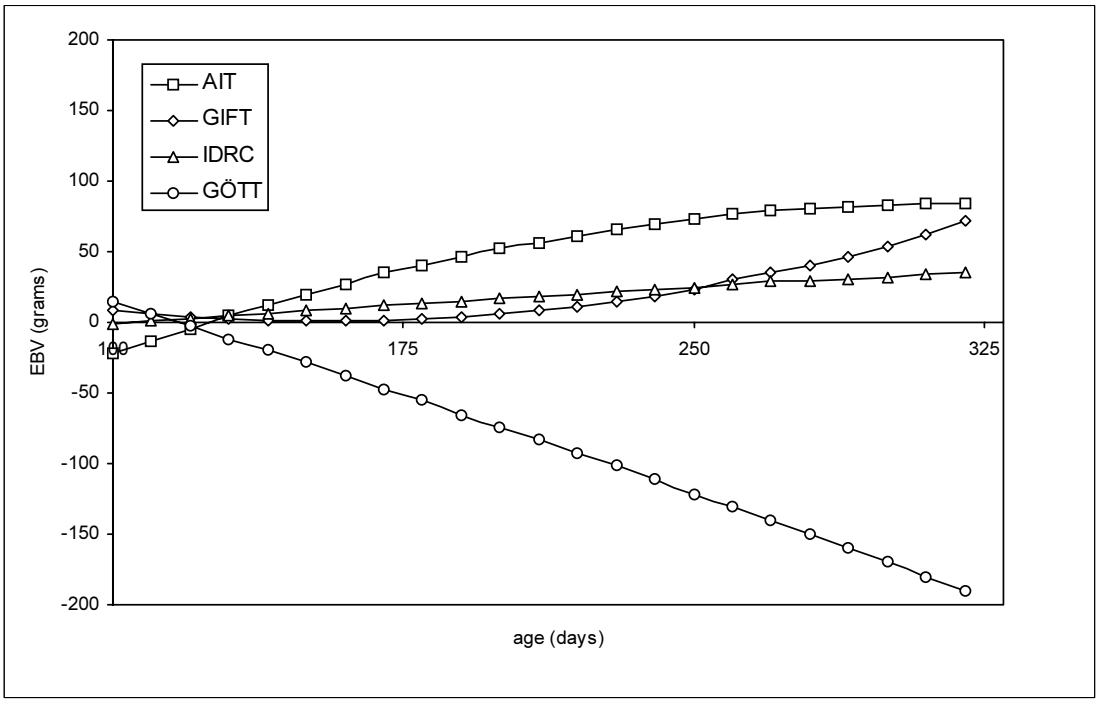

Figure 6. Average breeding values of all strains for body weight at different ages.

Discussion

Genetic analyses

Random regression models have been used frequently in livestock species, but in fish only by McKay et al. (2002). The estimation of genetic parameters using a random regression model was successful in this study. Estimates of variances and variance ratios were roughly comparable with estimates from the univariate model, although the family variance was somewhat lower when estimated by the random regression model. The heritability of body weight, one of the most important parameters in this respect, was fairly constant around 0.2. The magnitude of the heritability can be valued as moderate and although standard errors were high, due to large (additive genetic) variation, prospects for selection on body weight between 101 and 326 days post-hatching look promising. Genetic correlations were estimated between all ages (100 to 326 days) and are unique in their kind in Nile tilapia. At higher ages, genetic

correlations were clearly more stable: for example genetic correlations were over 0.9 between the age of 100 days and all ages up to 115 days of age; at higher ages genetic correlations were over 0.9 between the age of 223 days and all ages up to 326 days of age.

In our study two types of convergence problems were encountered. First, it was difficult to fit higher order (>second order) polynomial functions. When fitting uncorrelated (co)variance structures (i.e. no covariance's among random regression coefficients) to higher order models, convergence was achieved with somewhat more ease. However, estimates of variance components corresponding to the higher order terms were non-significant mostly. The eventual shape of the variance components (in time) of higher order models did not differ much (visually) from the second degree fit either. Although the (co)variance structure was different in the latter models, Apiolaza and Garrick (2001) showed that this does not have much impact on the shape and magnitude of the estimates of variance components. However, due to convergence problems in higher order models, uncertainty on the resulting estimates and uncertainty whether second order models were extended enough to fit the data could only be overcome by using an established method as the univariate model for comparison. Second, convergence problems occurred when incomplete records were retained in the data set. This was probably due to the information content of the family design. In general, convergence problems have been associated with random regression models before (Meyer, 1998).

Standard errors were high in this study (univariate analysis) due to the family structure. Standard errors were not available for the random regression model. These could be obtained by simulation or by using Markov Chain Monte Carlo (MCMC) methods to generate solutions to the model, but this was omitted in this study. Maternal half-sib families were present in this study, but maternal genetic effects could not be corrected for because there was insufficient depth in the pedigree. In full-sib families, possible variance due to maternal genetic effects could be absorbed by the random family effect. In maternal half-sib families, however, variance due to maternal effects could be absorbed by the additive genetic component. Consequently, the presented heritabilities might be biased upwards. The bias will depend on upon the magnitude of the maternal effects. Gall and Bakar (2002) showed that the maternal genetic variance as a proportion of the phenotypic variance in a cross of *Oreochromis mossambicus* and *O. hornorum* was low (0.02) and therefore the bias is expected to be small.

Comparison of the estimated genetic parameters with literature values was hard in this study. Genetic parameters are considered population and environment specific (Falconer and Mackay,

1996) and for that reason, no estimates from literature were valid for comparison although a number of studies are available. The estimates from literature varied from -0.10 to 0.47 for ages of 45 to 360 days (Tave and Smitherman, 1980; Langholz, 1987; Lester et al., 1988; Kronert et al., 1989; Oldorf et al., 1989; Eknath et al., 1998; Bolivar and Newkirk, 2002; Gall and Bakar, 2002). In livestock species, literature values can be used with somewhat more ease since populations have known genetic backgrounds and environments are often more comparable, but in fish this is rarely the case. Rutten et al. (2004b) indicated that it is very hard to obtain specific information on the genetic background of strains in Nile tilapia, and environments are hardly ever comparable because of differences in fish density, rearing system, feed and feeding levels etc.

In the current study, the heritability was fairly constant around 0.20 after the age of 150 days. Slight deviations in the pattern at low and high ages might be due to the amount of data present at these ages. The use of orthogonal polynomials to describe the effects included in the model can be another factor to explain deviations in the estimates, since polynomials are known to change direction easily outside the data interval. In general, the estimates from the random regression model can be viewed as more reliable compared to a univariate model. This is caused by the fact that a random regression model is less sensitive for changes in variances in time so that variance estimates are more accurate (Meyer, 1998).

Genetic parameters were estimated in a crossbred population in this study, but no correction factors for heterosis were included in the model. The strains used to compose the current population were found to be genetically distant (Rutten et al., 2004b), so the presence of effects of heterosis could be realistic in this experiment. Evidence for the presence of heterosis has been found before in Nile tilapia (Bentsen et al., 1998). In the current study, however, genetic groups for founder strains were used in the analysis, so the overall genetic level was properly adjusted for differences in the genetic level of founder stains. Furthermore, since heterosis was omitted in the model, possible effects of heterosis not adjusted for by genetic groups could be absorbed by the family effect.

In Nile tilapia breeding programs, full-sib families are often reared in a common environment for a certain period of time. Actually, mouth brooding can be considered as the first common environment. Later, this can be a rearing phase until tagging, or a rearing period until sufficient families are collected so that equal batches from families can be combined to a communal test in mass selection programs. Both Martínez et al. (1999) and Pante et al. (2002) stressed the

importance of including family effects in a model for variance estimation in fish. Omitting a family effect from the model, inflated additive genetic variances in both these studies. Model-terms to estimate family variance were included in both models in the current study, although the estimated magnitudes might have allowed omitting the terms from the model in some cases. In general, the ratio family variance to phenotypic variance (c^2) decreased in time, as was expected. The parameter shows, however, that effects of maternal-genetic, non-additive genetics or common environment can have impact for a long time on the performance of the animals. Efforts to equalize effects of rearing-environments early in life or correcting for the effect seem necessary therefore, when selection decisions are to be taken on the performance of animals.

Use of continuous genetic parameters

As shown in the results section, genetic parameters from a random regression model vary over time. Therefore, it can occur that selection on a correlated trait, say body weight at 200 days of age, can be more effective in terms of selection response than direct selection on the target trait, say, body weight at the age of 326 days. Results from a random regression model allow calculation of the age where (early) selection would result in the maximum selection response, using the formula to calculate correlated response: $CR_Y = i \cdot h_X \cdot h_Y \cdot r_g \cdot \sigma_{pY}$ (Falconer and Mackay, 1996), where i is the selection intensity, h is the square root of the heritability of the selection trait X and target trait Y, r_g is the genetic correlation and σ_{pY} is the phenotypic standard deviation of the target trait. This is illustrated in Figure 7, which shows that selection for improvement of body weight at the age of 326 days is optimal when the actual selection is based on body weight at the age of approximately 260 days. However, when the selection response is expressed as response per year, the optimal age of selection decreases even more which is due to a shortened generation interval. In our study the precision of genetic parameters was relatively low so these results should be interpreted with care. None the less, early selection can yield dramatic increases of selection response in Nile tilapia.

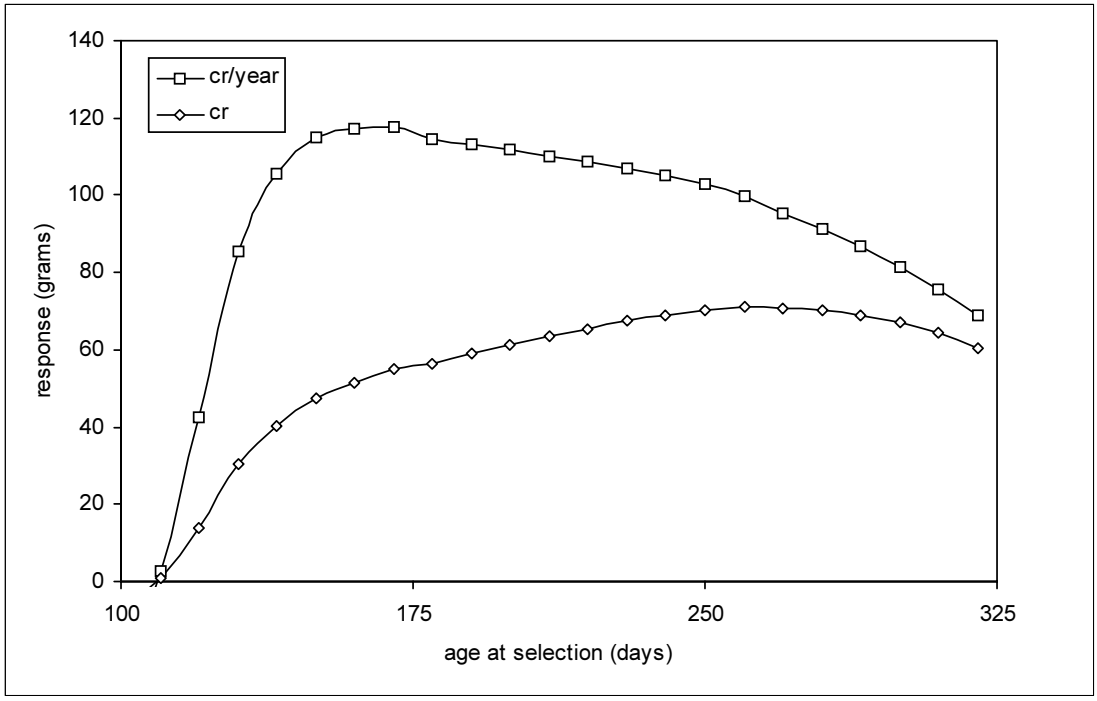

Figure 7. Correlated response (cr) from early selection and correlated response from early selection expressed per year (cr/year), where the target trait was body weight at the age of 326 days. Calculations were based on genetic parameters estimated with the random regression model*.

* Selection intensity was fixed at a value of 2 for simplicity, i.e. 5.7% of the animals were selected.

An interesting feature of the random regression model was the ability to identify average breeding values for body weight of strains at all ages. Not only differences in level but also differences in patterns were observed. This is the first time these differences are reported in literature, and they can have important implications for tilapia production. The strain of choice for use in a breeding program for example, should be taken cautiously, because it can affect the output of a farm to a large extend depending on the target market-weight. The efficiency of nutrient use combined with protein and fat physiology are important factors in this consideration, but little is known in this area. Generally weight gain in animals shifts from protein gain to fat gain as life progresses and therefore, fast development of body weight early in life is often more efficient (for extensive discussion on pigs see: Huisman, 2002). This study has shown that genetic variation for body weight patterns in Nile tilapia exists and methodology to utilize this genetic variation is available (Van der Werf, 2002). Consequently, implementation of a selection strategy to alter body weight curves is possible. Collecting repeated measurements of body weight in Nile tilapia, however, is not without consequence.

Netting fish is labor intensive and causes stress. Therefore this methodology does not seem very appealing to be applied routinely.

Male- versus female body weight

Although estimation of genetic parameters was the main aim of this paper, the phenotypic data showed some interesting features with respect to production of Nile tilapia. Eventually, five repeated observations of body weight of Nile tilapia were collected in this study. It turned out that average body weight of male and female fish was significantly different already at young ages (i.e. sexual dimorphism). At around 300 days, male fish had almost the double body weight of female fish, and with that, especially product uniformity is compromised when mixed sex populations are used for production. The origin of sexual-dimorphism has been attributed to behavioral factors (Schreiber et al., 1998), social interactions (Toguyeni et al., 2002), food deprivation in mouth-brooding female fish (Maar et al., 1966; Huet, 1972) and genetic factors (Fryer and Iles, 1972) in the past. In the additional bivariate genetic analysis, where body weight of male- and female fish were treated as separate traits, genetic correlations were generally high and not significantly different from unity. This means that body weight in male and female fish are most likely controlled by the same genes. Prospects to decrease the difference between mature male- and female body weight by selection are therefore unfavorable in Nile tilapia (Lande, 1980; Falconer and Mackay, 1996).

The average phenotypic body weight curves for all, male- and female fish showed a linear development in the last part of the trajectory and, especially for female fish, did not show a second bend towards a plateau. It has been mentioned often that female fish stop growing once sexual maturity has been reached, and from then onwards energy is spent for reproduction mainly. Fish-densities reached a maximum of 80 kg fish/m^3 in this experiment. These environmental circumstances were such that breeding and social interactions were probably suppressed, and this might be the reason that female fish continued to grow.

Evaluation of the general performance of the tested fish is difficult because comparable data of mature body weight was not available from literature. In one occasion, Langholz (1987) reported average body weights of 141 grams after 360 days (171 grams for male fish and 111 grams for female fish), but feeding was not *ad libitum* in that experiment. The performance presented in this study was not achieved in optimal conditions either. During the experiment, fish were netted and anaesthetized at least six times, which are both commonly regarded as very

stressful events in fish (Ruane, 2002). In addition, when fish were anaesthetized, they were deprived from food for one or two days. Consequently, the time to reach the average weight of 600 grams can be shortened, given more optimal conditions. Also, (test-) environments among experiments differ greatly and these can influence the development of body weight to a large extend (Oldorf et al., 1989; Bentsen et al., 1998).

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References

Apiolaza, L.A., Garrick, D.J., 2001. Analysis of longitudinal data from progeny tests: Some multivariate approaches. Forest Science 47, 129-140.

Bentsen, H.B., Eknath, A.E., Palada-de Vera, M.S., Danting, J.C., Bolivar, H.L., Reyes, R.A., Dionisio, E.E., Longalong, F.M., Circa, A.V., Tayamen, M.M., Gjerde, B., 1998. Genetic improvement of farmed tilapias: growth performance in a complete diallel cross expiriment with eight strains of *Oreochromis niloticus*. Aquaculture 160, 145-173.

Bijma, P., Arendonk, J.A.M. van, Woolliams, J.A., 2000. A general procedure to predict rates of inbreeding in populations undergoing mass selection. Genetics 154, 1865-1877.

Bolivar, R.B., Newkirk, G.F., 2002. Response to within family selection for body weight in Nile tilapia (*Oreochromis niloticus*) using a single-trait animal model. Aquaculture 204, 371-381.

Brzeski, V.J., Doyle, R.W., 1995. A test of an on-farm selection procedure for tilapia growth in Indonesia. Aquaculture 137, 219-230.

Eknath, A.E., Dey, M.M., Rye, M., Gjerde, B., Abella, T.A., Sevilleja, R., Tayamen, M.M., Reyes, R.A., Bentsen, H.B., 1998. Selective breeding of Nile tilapia for Asia. Proc. 6th World Congr. Genet. Appl. Livest. Prod. 27, 89-96.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to quantitative genetics. 4th ed. Pearson Education Limited, Harlow, UK.

Fryer, G., Iles, T.D., 1972. The cichlid fishes of the great lakes of Africa: Their biology and distribution. Oliver and Boyd, Edinburgh.

Gall, G.A.E., Bakar, Y., 2002. Application of mixed-model techniques to fish breed improvement: analysis of breeding-value selection to increase 98-day body weight in tilapia. Aquaculture 212, 93-113.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J., Thompson, R., 2002. ASReml User Guide Release 1.0. VSN International Ltd, 5 The Waterhouse, Waterhouse Street, Hemel Hempstead, HP1 1ES, UK

Huet, M., 1972. Breeding and cultivation of fish. Fishing News (Books) Ltd., Farnham, Surrey.

Huisman, A.E., 2002. Genetic analysis of growth and feed intake patterns in pigs. Doctoral thesis, Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences, Wageningen, The Netherlands.

Kronert, U., Horstgen-Schwark, G., Langholz, H.-J., 1989. Prospects of selecting for late maturity in Tilapia (*Oreochromis niloticus*) I. Family studies under laboratory conditions. Aquaculture 77, 113-121.

Lande, R., 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34, 292-305.

Langholz, H.-J., 1987. Intraspecific variation in tilapia. Proceedings of the 3rd status seminar, 27 april- 1 may, 1987, Tiberias, Israel. Special publication EAS, no 11, 1990., 103-118.

Lester, L.J., Abella, T.A., Palada, M.S., Keus, H.J., 1988. Genetic variation in size and sexual maturation of *Oreochromis niloticus* under hapa and cage culture conditions. Proceedings of the 2nd ISTA, 223-230.

Maar, A., Mortimer, M.A.E., Van der Lingen, I., 1966. Fish Culture in Central East Africa. FAO, Rome, Italy.

Martinez, V., Neira, R., Gall, G.A.E., 1999. Estimation of genetic parameters from pedigreed populations: lessons from analysis of alevin weight in Coho salmon (*Oncorhynchus kisutch*). Aquaculture 180, 223-236.

McKay, L.R., Schaeffer, L.R., McMillan, I., 2002. Analysis of growth curves in rainbow trout using random regression. Proc 7th WCGALP, Communication N° 06-11.

Meyer, K., 1998. Estimating covariance functions for longitudinal data using a random regression model. Genet. Sel. Evol. 30, 221-240.

Meyer, K., Hill, W.G., 1997. Estimation of genetic and phenotypic covariance functions for longitudinal or 'repeated' records by restricted maximum likelihood. Livest. Prod. Sci. 47, 185-200.

Nobre, P.R.C., Misztal, I., Tsuruta, S., Bertrand, J.K., Silva, L.O.C., Lopes, P.S., 2003. Analysis of growth curves of Nellore cattle by multiple-trait and random regression models. J. Anim. Sci. 81, 918-926.

Oldorf, W., Kronert, U., Balarin, J., Haller, R., Horstgen-Schwark, G., Langholz, H.-J., 1989. Prospects of selecting for late maturity in Tilapia (*Oreochromis niloticus*) II. Strain comparisons under laboratory and field conditions. Aquaculture 77, 123-133.

Pante, M.J.R., Gjerde, B., McMillan, I., Misztal, I., 2002. Estimation of additive and dominance genetic variances for body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 204, 383-392.

Quaas, R.L., 1988. Additive genetic model with groups and relationships. J. Dairy Sci. 71, 1338-1345.

Ruane, N.M., 2002. Stress and stress disorders in a teleost fish, the common carp *Cyprinus carpio* L. Doctoral thesis, Fish Culture and Fisheries Group, Wageningen Institute of Animal Sciences, Wageningen, The Netherlands.

Rutten, M.J.M., Bijma, P., Woolliams, J.A., Van Arendonk, J.A.M., 2002. SelAction: software to predict selection response and rate of inbreeding in livestock breeding programs. J. Heredity 93, 456-458.

Rutten, M.J.M., Bovenhuis, H., Komen, H., 2004a. Modeling fillet traits based on body measurements in three Nile tilapia strains (*Oreochromis niloticus* L.). Aquaculture 231, 113-122.

Rutten, M.J.M., Komen, H., Deerenberg, R.M., Siwek, M., Bovenhuis, H., 2004b. Genetic characterization of four strains of Nile tilapia (*Oreochromis niloticus* L.) using microsatellite markers. Animal Genetics 35, 93-97.

Schreiber, S., Focken, U., Becker, K., 1998. Individually reared female Nile tilapia (*Oreochromis niloticus*) can grow faster than males. J. Appl. Icht. 14, 43-47.

Tave, D., Smitherman, R.O., 1980. Predicted Response to Selection for Early Growth in Tilapia nilotica. Trans. Am. Fish. Soc. 109, 439-445.

Teichert-Coddington, D.R., Smitherman, R.O., 1988. Lack of Response by Tilapia nilotica to Mass Selection for Rapid Early Growth. Trans. Am. Fish. Soc. 117, 297-300.

Toguyeni, A., Fauconneau, B., Fostier, A., Abucay, J., Mair, G., Baroiller, J.-F., 2002. Influence of sexual phenotype and genotype, and sex ratio on growth performances in tilapia, *Oreochromis niloticus*. Aquaculture 207, 249-261.

Werf, J.H.J. van der, 2002. Optimising selection for traits along trajectories. Proc 7th WCGALP, Communication N° 16-07.

Westell, R.A., Quaas, R.L., Van Vleck, L.D., 1988. Genetic groups in an animal model. J. Dairy Sci. 71, 1310-1318.

Chapter VI

Is competitive behavior in Nile tilapia (*Oreochromis niloticus* L.) heritable?

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ABSTRACT

Debate whether selection for growth rate increases competitive behavior in fish has been going on for decades. All this time, the theoretical framework to answer this question was available, but never recognized. Now, methodology to estimate variance components for competitive genetic effects is available and applied to Nile tilapia. No genetic variation for competitive behavior was found although environmental variation for competitive behavior could clearly be detected. This methodology is potentially very interesting for breeding in aquaculture where effects of competition and aggression are very common, because no observations on behavior are required.

Introduction

Already for a long time, there is widespread concern that selection for growth rate in fish tends to select for more aggressive fish (Ruzzante and Doyle, 1991). The question whether this is true is still unanswered. Selection for growth rate can only increase competition when aggressive- or competitive behavior is heritable. A theoretical framework to answer this question has been available for decades (Griffing, 1967a), but methods enabling data analysis have only been developed recently (Muir and Schinckel 2002; Wolf, 2003).

In quantitative genetics, phenotypic observations are usually explained by a model where a phenotypic observation (P) is the sum of the population mean (μ), a random additive genetic component (A) and a random residual term (E): $P=\mu+A+E$. Many extensions to this model have been proposed to accommodate additional effects present in the data structure. However, the effect of interacting genotypes has mostly been ignored.

Around four decades ago, Griffing (1967a) argued that, when genotypes interact, the genetic model has to be extended to include not only a direct genetic effect of the individual itself, but also so called associate (genetic) effects due to group members (from this point onwards, "associate effects" are referred to as "competitive behavior" or "effects of competition"). Furthermore, Griffing (1967b) developed theory for predicting response to selection when genotypes interact, and showed that in this case selection on individual performance can surprisingly result in selection response in the opposite (undesired) direction. However, Griffing (1967b) also showed that when selection is based on group performance (i.e. "group selection", where entire groups are selected based on their mean group performance) rather

than on individual performance, selection response is always in the desired direction. Translation of this theory to practical animal production systems where animals are kept in groups means that group selection acts in a positive way on the direct genetic component, but at the same time acts negatively on the competitive behavior, i.e. group selection does not increase effects of competition. Eventually, group selection thus results in genetically better animals with respect to the trait of interest, and more "social" animals showing less competition among each other. Group selection (against cannibalism) has been successfully demonstrated in chickens (Muir, 1996) and quail (Muir and Schinckel, 2002).

In Nile tilapia, a clear hierarchical social structure develops when animals are reared within groups, and interactions between fish are common. This behavior is often observed when feed is provided, and when dominant fish try to maintain a territorial area. However, it also occurs for less obvious reasons and can have severe implications including death of the repressed animal. This kind of behavior is common in many fish species and literature is available in abundance. When genetic variation for associate effects is present in Nile tilapia, group selection seems a promising strategy improve behavior while selecting for improved performance at the same time.

This study, therefore, investigates whether genetic variation for competitive behavior is present in Nile tilapia. A recently proposed variance estimation procedure (Muir and Schinckel, 2002) was adopted to estimate variance components. Whether or not group selection has the potential to simultaneously increase production performance and decrease competition in Nile tilapia is discussed.

Materials and methods

Experiment

Eight dams and 8 sires were used to produce 8 full-sib families for this experiment. The parents originated from a 1st generation cross of four Nile tilapia strains (for details on strains see: Rutten et al., 2004). Initially, eggs and semen were collected by hand-stripping and artificially incubated after fertilization. After hatching, 200 larvae per family were stocked in 8 separate tanks (20 l.), and reared for 2 months with *ad libitum* feeding. All families were produced within 2 days.

After initial rearing, 60 fingerlings per family were tagged using Floy® tags. At the start of the experiment, 450 animals of 8 full-sib families were randomly allocated to 45 tanks. Fingerlings that died within the first day were replaced by family members.

Individual body weight was recorded at the start of the experiment and at the end. The experiment lasted 6 weeks. The amount of feed provided was based on the average body weight of all animals at the start of the experiment. Subsequently, each 2nd week, average weight of all animals was recorded and the amount of feed was adjusted accordingly. For one tank of fish, the amount of feed per day was calculated as $0.03*BW_A*10$, where BW_A denotes average body weight of all fish. The total amount of feed per day was provided in three parts by point feeding. At the end of the experiment the fish were approximately 100 days of age.

Variance component estimation

In the classical quantitative genetic model, a phenotypic observation (*P*) is explained by an additive genetic component (*A*) and a random residual component (*E*): $P=A+E$ (Falconer and Mackay, 1996). When animals are kept in groups, social interactions among them may affect their phenotypes. Social behavior may have a genetic component which can be selected for. When social interactions occur, the phenotype of an animal (P_i) in a group of size *n*, can be modeled as the sum of its own direct phenotypic effect ($P_{d,i}$) plus *n-1* phenotypic effects of group mates ($P_{a,j}$; Griffing, 1967a). Note, that $P_{d,i}$ and $P_{a,j}$ are not observable. Both the direct phenotypic effect and the phenotypic effects of group mates can be divided into an additive genetic- (*A*) and a residual (*E*) component (Griffing, 1967a; Muir and Schinckel, 2004):

$$P_i = P_{d,i} + \sum_{j=1}^{n-1} P_{a,j} = A_{d,i} + E_{d,i} + \sum_{j=1}^{n-1} \left(A_a + E_a\right)_j$$

where, *i* denotes the individual itself and *j* denotes one of the *n-1* group members. From the model follows that the phenotypic variance is:

$$\sigma_P^2 = \sigma_{P_d}^2 + (n-1)\sigma_{P_a}^2 = \sigma_{A_d}^2 + \sigma_{E_d}^2 + (n-1)(\sigma_{A_a}^2 + \sigma_{E_a}^2) \quad [1]$$

when group members are unrelated. Note, that in contrast to the classical quantitative genetic model, the phenotypic variance depends on group size *n* because the group size influences the magnitude of the variance due to group mates.

Within groups, phenotypes of two animals are correlated because: 1) in groups of size $n>2$, the phenotypes of each of the two group members are affected by the phenotypes of the same *n-2* other group members, and 2) the direct effect and the competitive effect of an animal may be

correlated, which causes a correlation between the phenotype of the animal itself and that of its group member. The covariance between phenotypes of two group members *i* and *j* can, according to the genetic model, be written as:

$$Cov(P_i, P_j) = 2\sigma_{P_{d,a}} + (n-2)\sigma^2_{P_a} = 2(\sigma_{A_{d,a}} + \sigma_{E_{d,a}}) + (n-2)(\sigma^2_{A_a} + \sigma^2_{E_a}) \quad [2]$$

(for derivation see: Appendix A)

when animals are unrelated. When, however, related animals are in the same group, the following terms have to be added to equation [2]:

$$a\sigma^2_{A_d} + 2(n-2)a\sigma_{A_{d,a}} + [(n-1)^2 - (n-2)]a\sigma^2_{A_a}.$$

Note, that both the additive genetic- ($\sigma_{A_{d,a}}$) and residual ($\sigma_{E_{d,a}}$) covariances between direct and competitive effects are present in this expression. In a standard (animal) genetic model for estimation of genetic parameters, the numerator relationship matrix (Henderson, 1975) accommodates all additive genetic covariances between individuals and residual terms are usually modeled independently for each observation. With social interactions, however, covariances between individuals are partly caused by non-genetic terms, as can be seen from equation [2]. Those covariances are not accommodated by the numerator relationship matrix. Therefore, a special residual structure is required to accommodate those non-genetic covariances within-, but not between tanks. In matrix notation, the model for estimation of variance components is:

$$y = Xb + Z_d a_d + Z_a a_a + \varepsilon$$

where, *y* is an observation of body weight at the end of the experiment, *b* is a vector for fixed effects which accommodated only the general mean, a_d and a_a are additive genetic values for the direct- and social effects respectively, *X*, Z_d and Z_a are incidence matrices and ε is a random residual term. The model has expectations:

$$E(y) = Xb \text{ and } E(a_d) = E(a_a) = E(\varepsilon) = 0$$

and variance structure:

$$V\begin{pmatrix} a_d \\ a_a \\ \varepsilon \end{pmatrix} = \begin{pmatrix} A\sigma^2_{A_d} & A\sigma_{A_{d,a}} & 0 \\ A\sigma_{A_{d,a}} & A\sigma^2_{A_a} & 0 \\ 0 & 0 & R\sigma^2_{\varepsilon} \end{pmatrix}$$

where *A* is the numerator relationship matrix and *R* is a correlation matrix of residual effects with $R_{ii}=1$ on the diagonal, $R_{ij}=\rho$ within tanks and $R_{ij}=0$ between tanks. Hence, *R* is block-diagonal.

From equations [1] and [2] follows that the correlation (ρ) between residual effects of tank mates equals:

$$\rho = \frac{2\sigma_{E_{d,a}} + (n-2)\sigma^2_{E_d}}{\sigma^2_{E_d} + (n-1)\sigma^2_{E_a}}$$

and this correlation is estimated. Potential tank effects are absorbed within this correlation. Furthermore, $\sigma^2_{E_d}$, $\sigma^2_{E_a}$ and $\sigma_{E_{d,a}}$ are not separately identifiable; only their combined effect, which surfaces as correlated residuals within tank, can be estimated. The model does not require any specific experimental design, i.e. groups may be composed of arbitrary animals. To verify the results of the model including effects of competition, a (classical) model was used where a direct additive effect and a random tank effect were modeled. Genetic groups were included to account for genetic differences between strains (Westell et al. 1988; Quaas, 1988) and variance components were estimated using restricted maximum likelihood (REML) implemented in ASReml software (version 1.10, 15 oct 2003; Gilmour et al., 2002).

Results

Table 1 shows the numbers of observations available for analysis. In 28 tanks, all animals survived to the end of the experiment, which resulted in 280 records on body weight. In 13 tanks one animal died during the experiment which resulted in 117 records. In 4 tanks, 2 up to 4 animals died during the experiment. In total, 25 animals out of 450 died during the experiment which is around 5.5%.

Table 1. Overview of the number of tanks with the number of animals and the resulting total number of observations at the end of the experiment.

n Animals	n Tanks	n Observations
10	28	280
9	13	117
8	1	8
7	2	14
6	1	6
total	45	425

The distribution of animals over families is presented in Table 2. Families contributed from 48 (fam 3) up to 66 (fam 4) animals. Depending on family, the fish were either 108, 109 or 110

days of age when the final measurements of body weight were taken. Average weight per family, at the start of the experiment, varied from 3.7g (fam 8) to 7.3g (fam 1). At the end of the experiment, the average weight per family varied from 44.5g (fam 3) to 66.8g (fam 2). The

Table 2. Number of animals, average weight at the start- and the end of the experiment and the number of animals that died specified per family.

	Start of experiment		End of experiment		Died
Family	n	mean weight	n	mean weight	n
1	56	7.3	55	53.5	1
2	55	5.9	53	66.8	2
3	48	5.4	45	44.5	3
4	66	4.3	60	51.5	6
5	54	4.6	53	54.5	1
6	58	5.7	56	55.6	2
7	58	4.4	53	61.9	5
8	55	3.7	51	55.0	4

ranking of families changed from the start to the end of the experiment. For example, family 1, which showed the highest average weight at the beginning of the experiment, showed the third lowest average weight at the end of the experiment. The number of animals that died during the experiment varied from 1 to 6 per family. The correlation between average family weights at the start of the experiment with the number of animals from that family that died during the experiment was -0.67, which means that the mortality in a family was high when the average family weight was low.

Estimated genetic parameters are presented in Table 3. Using the model including only a direct genetic effect, the variance due to tanks was close to zero and therefore omitted from the model. The additive genetic variance was 106.7g^2, and the error variance 154.1g^2. The phenotypic variance, the sum of the former two components, was 260.8g^2 and consequently the heritability of the direct genetic effect was 0.41. The standard error of the heritability was relatively high (0.25) and ρ was -0.073.

Using the model including both a direct genetic- and an competitive genetic effect, the direct genetic variance, residual variance and phenotypic variance were 107.0g^2, 152.2g^2 and 259.2g^2 respectively. The genetic variance due to competitive behavior was estimated to be close to

zero. The heritability of the direct genetic effect was 0.41 again, with a standard error of 0.26 and ρ was -0.076.

Table 3. Additive genetic variance for the direct effect (σ^2_{Ad}), additive genetic variance for the competitive effect (σ^2_{Aa}), residual variance (σ^2_e), phenotypic variance (σ^2_P), heritability of the direct effect (h^2_d), genetic correlation between direct and competitive effects (r_g) and within tank residual correlation (ρ) for models including only a direct genetic effect (D) and with both a direct genetic- and a competitive genetic effect (DA).

Parameter	D	DA
σ^2_{Ad}	106.7±82.1	107.0±82.9
σ^2_{Aa}	-	0.000
σ^2_e	154.1±43.6	152.2±44.1
σ^2_P	260.8±43.4	259.2±43.8
h^2_d	0.41±0.25	0.41±0.26§
r_g	-	n.e.‡
ρ	-0.073±0.03	-0.076±0.03

§ Heritabilities from the model with associate effects are not defined as in the classical context.

‡ Not estimable because $\sigma^2_{Aa}=0$.

Discussion

Studies on aggression and cannibalism in fish, and Nile tilapia in particular, are numerous (e.g. Berrios-Hernandez, 1983; Pantastico et al., 1988; Smith and Reay, 1991; Hecht and Pienaar, 1993; Giaquinto and Volpato, 1997; Baras and Jobling, 2002). However, strategies to overcome behavior of aggression and cannibalism among fish were always sought in environmental solutions. Recently, Muir (1996) and Muir and Schinckel (2002) have shown experimentally that this behavior can be altered genetically in layers (*Gallus gallus*) and quail (*Coturnix japonica*) respectively. This result was achieved by using group selection for several generations, in which case genetic parameters do not need to be estimated.

Disentangling phenotypic variance caused by behavior of aggression and cannibalism into a genetic component and an environmental component has only been done in fruit fly (*Drosophila melanogaster*; Wolf, 2003) and pigs (*Sus scrofa*; Cassady and Van Vleck, 2004) to our knowledge. Cassady and Van Vleck (2004) reported very low heritabilities of competitive effects for the number of days to reach 105kg live weight (0.01), average daily gain (0.02) and amount of backfat adjusted to 105kg live weight (0.00). Wolf (2003) reported a heritability of competitive

effects on body size (0.18). In our study, no evidence was found that competitive effects on body weight have a genetic background in Nile tilapia. Based on this result, it does not seem likely that quantitative genetics can have a contribution in improving these effects in Nile tilapia.

The heritability of the direct effect was high in this study (0.41) compared to other estimates in the same population (Rutten et al., 2005). Although this was not the primary interest of this study, it can give an indication of the appropriateness of the model. The magnitude of the heritability was caused by the fact that no effect common to full-sibs was modeled in this study (Martinez et al., 1999; Pante et al., 2002). When both an additive genetic and a full-sib effect were present in the model, a constraint on one of both variance components was required to prevent it from inflating and thus absorbing variance due to both effects. With constraints, the variance components for direct additive genetic- and full-sib variance were similar to estimates presented by Rutten et al. (2005; not shown).

The methodology used in this study is quite recent; therefore, it is still unclear how to best deal with aspects like e.g. animals that die during the experiment. Often, maybe especially in fish, it is impossible to determine the cause of death of an animal. Continuous interactions between fish preceding the death of an animal were never observed in our experiment. When, however, the death of an animal is caused by effects of competition, some way of correction should probably be included in the analysis. In that case, animals that died are informative for the behavior of tank mates. In our study, two ways to correct for this were tried: 1) a covariate for the remaining number of animals in the tank was included in the model, and; 2) the phenotypic values of animals that died were given the lowest value present in the data. However, both ways of correction had little effect on the estimated parameters (not shown). Further investigations into this methodology may provide insight in how to deal with this problem in the future.

Although no evidence was found for the presence of a genetic component in competitive behavior, non-genetic competitive behavior was detected, which is indicated by the within environment correlation of -0.076. The interpretation of the magnitude of this correlation with respect to the severity of competitive behavior, however, is hard. The fish within this experiment were not fed *ad libitum*, yet, fierce effects of competition for feed were never observed. In Nile tilapia, it is known that very fierce competition can take place very early in life especially between members of different families (Giaquinto and Volpato, 1997) which often results in the death of animals. The animals that were used in this experiment were all reared

within full-sib families, where effects of competition and aggression are milder. Therefore, the animals that would have been killed when full-sib families are combined into one environment at an earlier stage, as is usually the case in intensive aquaculture, had the chance to participate in this experiment and therefore maybe even more severe effects of competition were expected.

Quantitative genetic research in fish has contributed very little to insight into competitive behavior. Doyle and Talbot (1986) showed, using game theory, that it is highly unlikely that selection for rapid growth indirectly selects for aggressive fish. The reasoning behind this was that competition is not necessary in non-natural environments where enough food is available. Fish that spend less energy on competitive behavior are more efficient, and will therefore grow faster and enhance their chance of mating success. Ruzzante and Doyle (1991) selected medaka fish (*Oryzias latipes*) in two environments of low and high intensity of interactions between fish. An indication was found that domestication selection favors fish that are more social. After other experiments with medaka, Ruzzante and Doyle (1993) supported the hypothesis reported by Doyle and Talbot (1986) that an environment where competition is present favors the more tolerant fish. Vøllestad and Quinn (2003) recorded aggressive behavior in Coho salmon (*Oncorhynchus kisutch*) and subsequently performed a genetic analysis which resulted in a (non-significant) heritability of 0.250. They found a (non-significant) negative genetic correlation (-0.36) between growth rate and agonistic behavior, implying that selection for growth rate in Coho salmon can possibly decrease agonistic behavior due to a correlated response.

The theory of group selection was developed by Griffing (1967b) as a means to improve both direct performance and associate behavior simultaneously. In that respect, the current mixed model methodology has several advantages over group selection. In group selection, the amount of selection response is completely depending on the genetic parameters and selection intensity. When mixed model methodology is used to estimate BLUP breeding values, however, a selection index including breeding values for both direct and associate genetic effect can be designed and selection response in both traits can be directed by altering the weights given to breeding values for both traits. Furthermore, when improvement of competitive behavior is desired and a genetic component can be identified, very labor intensive methods as scoring behavior on individual fish (Ruzzante and Doyle, 1991, 1993; Vøllestad and Quinn, 2003) can be avoided. Therefore, this methodology is potentially very interesting for fish breeding, because effects of competition and cannibalism/aggression are still very common in aquaculture and highly undesirable.

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References

Baras, E., Jobling, M., 2002. Dynamics of intracohort cannibalism in cultured fish. Aquacult. Res. 33, 461-479.

Berrios-Hernandez, J.M., Snow, J.R., 1983. Comparison of methods for reducing fry losses to cannibalism in tilapia production. The Progressive Fish-Culturist 45, 116-118.

Cassady, J.P., Van Vleck, L.D., 2004. Estimation of (co)variance componenets duet o genetic competition effects in pigs. J. Anim. Sci. 82 Suppl. 2, N° 25.

Doyle, R.W., Talbot, A.J., 1986. Artificial selection on growth and correlated selection on competitive behavior in fish. Can. J. Fish. Aquat. Sci. Vol. 43, 1059-1064.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to quantitative genetics. 4th ed. Pearson Education Limited, Harlow, UK.

Giaquinto, P.C., Volpato, G.L., 1997. Chemical communication, aggression and conspecifics recognition in the fish Nile tilapia. Physiol. & Behav. 62, 1333-1338.

Gilmour, A.R., Gogel, B.J., Cullis, B.R, Welham, S.J., Thompson, R., 2002. ASReml User Guide Release 1.0. VSN International Ltd, 5 The Waterhouse, Waterhouse Street, Hemel Hempstead, HP1 1ES, UK

Griffing, B., 1967a. Selection in reference to biological groups. I. Individual selection applied to populations of unordered groups. Aust. J. Biol. Sci. 20, 127-139.

Griffing, B., 1967b. Selection in reference to biological groups. IV. Application of selection index theory. Aust. J. Biol. Sci. 22, 131-142.

Hecht, T., Pienaar, A.G., 1993. A review of cannibalism and its implications in fish larviculture. J. World Aquaculture Soc. 24, 246-261.

Henderson, C.R., 1975. A rapid method for computing the inverse of a relationship matrix. J. Dairy Sci. 58, 1727-1730.

Martinez, V., Neira, R., Gall, G.A.E., 1999. Estimation of genetic parameters from pedigreed populations: lessons from analysis of alevin weight in Coho salmon (*Oncorhynchus kisutch*). Aquaculture 180, 223-236.

Muir, W.M., 1996. Group selection for adaptation to multiple-hen cages: Selection program and direct responses. Poultry Sci. 75, 447-458.

Muir, W.M., Schinckel, A., 2002. Incorporation of competitive effects in breeding programs to improve productivity and animal well being. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, August 19-23, 2002, Montpellier, France. Communication N° 14-07.

Pantastico, J.B., Dangilan, M.M.A., Eguia, R.V., 1988. Cannibalism among different sizes of tilapia (*Oreochromis niloticus*) fry/fingerlings and the effect of natural food. Proceedings of the Second International Symposium on Tilapia in Aquaculture, 465-468.

Pante, M.J.R., Gjerde, B., McMillan, I., Misztal, I., 2002. Estimation of additive and dominance genetic variances for body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 204, 383-392.

Quaas, R.L., 1988. Additive genetic model with groups and relationships. J. Dairy Sci. 71, 1338-1345.

Rutten, M.J.M., Komen, H., Deerenberg, R.M., Siwek, M., Bovenhuis, H., 2004. Genetic characterization of four strains of Nile tilapia (*Oreochromis niloticus* L.) using microsatellite markers. Animal Genetics 35, 93-97.

Rutten, M.J.M., Komen, H., Bovenhuis, H., 2005. Longitudinal genetic analysis of Nile tilapia (*Oreochromis niloticus* L.) body weight using a random regression model. Aquaculture, in press.

Ruzzante, D.E., Doyle, R.W., 1991. Rapid behavioral changes in medaka (*Oryzias latipes*) caused by selection for competitive and noncompetitive growth. Evolution 45, 1936-1946.

Ruzzante, D.E., Doyle, R.W., 1993. Evolution of social-behavior in a resource-rich, structured environment: Selection experiments with medaka (*Oryzias latipes*). Evolution 47, 456-470.

Smith, C., Reay, P., 1991. Cannibalism in teleost fish. Rev. Fish Biol. Fisheries 1, 41-64.

Vøllestad, L.A., Quinn, T.P., 2003. Trade-off between growth rate and aggression in juvenile coho salmon, *Oncorhynchus kisutch*. Anim. Behav. 66, 561-568.

Westell, R.A., Quaas, R.L., Van Vleck, L.D., 1988. Genetic groups in an animal model. J. Dairy Sci. 71, 1310-1318.

Wolf, J.B., 2003. Genetic architecture and evolutionary constraint when the environment contains genes. Proc. Natl. Acad. Sci. USA 100, 4655-4660.

Appendix A

Derivation of $Cov(P_i, P_j)$ according to the assumed genetic model:

$$P_i = P_{d,i} + \sum_{j=1}^{n-1} P_{a,j} = A_{d,i} + E_{d,i} + \sum_{j=1}^{n-1} \left(A_a + E_a\right)_j \quad [A1]$$

In the derivation of $Cov(P_i, P_j)$ we assume that groups consist of *n=3* animals for convenience. Subscripts *i*, *j* and *k* denote the three group members. Then,

$$Cov(P_i, P_j) = Cov(P_{d,i} + \sum_{m=j,k} P_{a,m}, P_{d,j} + \sum_{m=i,k} P_{a,m}) \quad [A2]$$

$$Cov(P_i, P_j) = Cov(A_{d,i} + E_{d,i} + \sum_{m=j,k} A_{a,m} + \sum_{m=j,k} E_{a,m}, A_{d,j} + E_{d,j} + \sum_{m=i,k} A_{a,m} + \sum_{m=i,k} E_{a,m}) \quad [A3]$$

Splitting up equation A3, results in:

$Cov(A_{d,i}, A_{d,j}) = a\sigma^2_{A_d}$, where *a* denotes the additive genetic relationship of animals *i* and *j*,

$2Cov(A_{d,i}, E_{d,j}) = 0$

$2Cov(A_{d,i}, \sum_{m=i,k} A_{a,m}) = 2(\sigma_{A_{d,a}} + (n-2)a\sigma_{A_{d,a}})$

$2Cov(A_{d,i}, \sum_{m=i,k} E_{a,m}) = 0$

$Cov(E_{d,i}, E_{d,j},) = 0$

$2Cov(E_{d,i}, \sum_{m=i,k} A_{a,m}) = 0$

$2Cov(E_{d,i}, \sum_{m=i,k} E_{a,m}) = 2\sigma_{E_{d,a}}$

$Cov(\sum_{m=j,k} A_{a,m}, \sum_{m=i,k} A_{a,m}) = (n-2)\sigma^2_{A_a} + (n-1)^2 - (n-2)a\sigma^2_{A_a}$

$2Cov(\sum_{m=j,k} A_{a,m}, \sum_{m=i,k} E_{a,m}) = 0$

$Cov(\sum_{m=j,k} E_{a,m}, \sum_{m=i,k} E_{a,m}) = (n-2)\sigma^2_{E_a}$

Chapter VII

General discussion

The objective of this study was to investigate aspects enabling the design of breeding programs, to improve production of Nile tilapia reared under intensive conditions. In the General introduction of this thesis, the steps involved in the design and implementation of a breeding program were listed. In Chapters II to VI of this thesis, research was presented which provides information to enable the design of breeding programs for Nile tilapia under intensive circumstances. Finally, in this Chapter, aspects of breeding programs for Nile tilapia are further discussed and possibilities for future developments are presented.

Selection

Selection strategy

Some decades ago when interest in fish breeding started to develop, breeding programs for fish were mostly based on a combination of within- and between family selection. In family selection, all families are kept separately but the differences lie in the procedure of selection. In between family selection, a few of the best families are selected completely, based on their average phenotypic performance. In within family selection, only a few of the best animals within each family are selected. In combined within- and between family selection, a few of the best animals within only a limited number of families are selected. Family selection was advocated because the relative selection response from forms of family selection (within and between) is higher compared to mass selection, especially at low heritabilities (Gjedrem, 1983). In mass selection, all animals are kept in one environment and the best animals are selected based on their own phenotypic performance. However, to determine whether mass- or family

selection is more effective, other aspects play a role as well. When e.g. common environmental effects are present, the relative advantage of within family selection over between family selection and mass selection, increases. In Chapters IV and V it was shown that common environmental effects are present in Nile tilapia, although variance components were not always significantly different from zero. However, both Martinez et al. (1999) and Pante et al. (2002) stressed that common environmental effects should always be included in models for variance components estimation in fish, because additive genetic variances might be overestimated otherwise. Additional analyses using the data in Chapters IV and V, and a model where a common environmental component was omitted, confirmed this. Furthermore, it is important to evaluate what the requirements are to sufficiently control inbreeding using one of the mentioned selection strategies. Between family selection often results in high rates of inbreeding, whereas inbreeding is easily controlled in within family selection. For mass selection strategies in fish, methodology has been developed to design breeding programs, given a predetermined rate of inbreeding (Villanueva et al., 1996). In general, formulae for all kinds of selection strategies have been developed recently to predict the rate of inbreeding from breeding programs (Bijma, 2000).

In Nile tilapia, both mass selection (Tave and Smitherman, 1980; Hulata et al., 1986; Teichert-Coddinton and Smitherman, 1988), within family selection (Bolivar and Newkirk, 2002) and combined within- and between family selection (the GIFT project; Dey and Gupta, 2000) have been used as selection strategies to improve body weight. With exception of the GIFT breeding program, these strategies were merely used to generate a few generations of selection response so that subsequently genetic parameters could be estimated. Few recommendations have been made with respect to the most efficient selection strategy for (Nile) tilapia breeding programs. Uraiwan and Doyle (1986) showed that within family selection would probably result in the highest selection response in Nile tilapia; however, these results were generated given the specific facilities of their institute. Gall and Bakar (2002) argued that the most efficient method should be used for selection in tilapia which is BLUP selection (Wray and Hill, 1989; Villaneuva et al., 1993). However, recently Sonesson et al. (2005) showed that BLUP selection might result in unacceptable high rates of inbreeding in fish breeding programs and that therefore mass selection is more desirable.

The decision on what selection strategy should be used, depends heavily on the available facilities, the costs to operate these facilities and further costs necessary to enable a certain

selection strategy e.g. labor and tags etc. However, in literature, no special attention seems to be given to these aspects. Within Chapters III and IV of this thesis, the starting point was to investigate aspects of Nile tilapia breeding which enable the use of a mass selection strategy because of its relative simplicity, which would suit starting breeding programs. In this Chapter, advantages and disadvantages of various selection strategies are explored and discussed in terms of the necessary facilities, workload, selection response and rate of inbreeding, in relation to the research presented in this thesis. The discussion is led from the starting point that the breeding goal consists of only one trait, say, body weight. Later on, it is discussed what the consequences are when the breeding goal is extended to include more traits.

Facilities

When considering facilities necessary for various selection strategies, we can distinguish roughly two phases in Nile tilapia: an early rearing phase and a later fattening phase. Nile tilapia spawn unsynchronized and uncontrolled, and the number of eggs within one spawn can vary tremendously (Coward and Bromage, 2000). To be able to combine fry of different families and different ages to one (test) cohort, differences in body size among families cannot be too big in order to prevent cannibalism (Pantastico et al., 1988; Smith and Reay, 1991; Hecht and Pienaar, 1993). Relative size differences decrease with time in Nile tilapia, so when families of different ages are reared separately for a certain period of time, the relative size differences decrease, and risk of cannibalism is avoided (Fessehaye et al., 2004). Therefore, separate tanks are necessary to accommodate different families in the early rearing phase. Later on, large differences in body size among families could result in effects of competition (Chapter VI). Therefore, only larvae produced in a limited period of time can be combined and the capacity of the facilities for early rearing can be kept within a certain limit. In order to prevent confounding of effects of families with effects of tanks, certain measures have to be taken, such as a half-sib family structure or repetitions in the allocation of families to tanks.

In Table 1, a summary of facilities necessary to enable three selection strategies is presented. It shows that mass selection programs are straightforward in the sense that all selection candidates can be reared in one environment. After the initial rearing phase in Nile tilapia, only one tank is necessary and selection candidates can be reared e.g. simultaneously with other batches of fish within the normal production cycle. Moreover, in this way the environmental circumstances for

selection candidates are similar to the environmental circumstances for production fish and thus the same traits are measured.

In the case of family selection, separate tanks have to be available for each family. These tanks have to be big enough to accommodate families until the end of the testing period. However, probably several tank-sizes will have to be available to be able to adjust fish densities per tank during the rearing period.

To enable BLUP selection, basically the same facilities as for mass selection are necessary. The facilities to accommodate fry would have to be big enough to accommodate families until fish can be individually tagged. When fish are reared to high body weights as for the European market, the chance of losing external tags as e.g. Floy® tags seems be to be quite large (Bentsen et al., 1998; Chapter V), so a better solution would be to use PIT tags. These can, however, only be inserted at higher body weights compared to Floy® tags (approximately 50 grams

Table 1. Facilities necessary to perform mass- family- and BLUP selection strategies in Nile tilapia specified for an early rearing phase and a later fattening phase.

Selection strategy	Necessary facilities	
	Early rearing phase	Fattening phase
Mass selection	Separate aquaria for each family until the start of communal rearing (±1 month)	1 communal environment
Family selection	Separate aquaria for each family	Separate aquaria for each family (replicates?)
BLUP selection	Separate aquaria for each family until PIT tagging (±50 grams=±3 months)	1 communal environment

which takes around 3 months to reach under intensive circumstances; Chapter VI). After tagging, the fish are preferably kept in one communal testing environment. To enable the calculation of BLUP breeding values, a structured data collection system is necessary. In addition, a BLUP selection strategy requires a structured data collection system a genetic expertise to interpret results etc.

Workload

A summary of the necessary workload to enable the discussed selection strategies is presented in Table 2. The workload necessary to operate a breeding program depends on the scale of the breeding program. In this section, however, workload necessary to operate a certain selection strategy is discussed on a relative basis.

Table 2. Necessary work and relative work load in mass- family- and BLUP selection programs. Relative workload is expressed in comparison to the same work in other selection strategies

Selection strategy	Necessary work	Relative work load
Mass selection	*Standardize family sizes	o
	*Maintain 1 large tank	o
	*Collect measurements and select fish	o
Family selection	*Standardize family size	o
	*Maintain several smaller tanks	++
	*Collect measurements and select fish	+
BLUP selection	*Standardize family size and tag each individual fish	+++
	*Maintain 1 large tank	o
	*Collect measurements, genetic evaluation and select fish	+

In mass selection programs, families have to be collected and subsequently many tanks have to be maintained during initial rearing. When (test) cohorts are composed for communal rearing, the number of fingerlings per family has to be restricted so that each family is equally represented at the start. In this way, fish from each family have the same chance of being selected and rapid increase of inbreeding can be avoided. When the test cohort is stocked, the workload is relatively low, because only one tank has to be maintained. Later, at the time of selection, a sample has to be taken to determine the mean and standard deviation for the trait of selection in both sexes. Subsequently, all fish have to be weighed and selected based on truncation-points provided by the distribution using the sampled means and standard deviations.

In family selection programs, the initial rearing phase workload is the same as in mass selection programs. After the initial rearing phase, however, families have to stay separated from each other, and thus many tanks have to be maintained. Moreover, to be able to correct for tank- or family effects, combinations of families or half-sib structures or replicates are necessary. At the moment of selection, all fish have to be weighed. Depending on the form of family selection (between, within etc.), weighing has to be done twice in some occasions, once to determine average family weight, once to determine individual weight.

When BLUP selection is used, the workload is essentially the same as in mass selection programs until the moment that test-cohorts are composed in mass selection programs. At that moment, fish are too small to tag using PIT tags. Thus, families have to be reared to higher body weights in separate tanks, which means that more tanks have to be maintained. The next step would be inserting PIT tags into each individual fish which is very labor intensive work. After tagging, fish can be reared further in one environment. At the end of this rearing period, each fish has to be weighed so that data is collected and BLUP breeding values can be estimated. Then each individual fish has to be caught again, so that the best ones can be selected based on their estimated BLUP breeding values. To reduce the amount of work, it might be possible to make a pre-selection of the best fish phenotypically because these fish are likely to have the better breeding values. Compared to family selection, workload in BLUP selection programs is concentrated mainly around the moment of tagging and –selection, whereas workload in family selection is expressed in the maintenance of many tanks which is spread over the total rearing period.

Selection response

Selection response from the discussed selection strategies is presented in Table 3. Mass selection was used as a basic scenario here and the computer program SelAction (Rutten et al., 2002) was used to calculate selection response from different selection strategies. A population size of 10000 animals was assumed and each generation 50 sires and 100 dams were selected. Given the genetic parameters (see: Table 3), 64.6g selection response can be achieved each generation using mass selection, while the coefficient of inbreeding increases by 0.8% per generation. SelAction (Rutten et al., 2002) allowed to calculate selection response from either between family selection or combined within- and between family selection but not within family selection. Table 3 shows that both forms of family selection result in unacceptably high

rates of inbreeding (10% and 5% per generation). Generally, 1% increase of inbreeding per generation is acceptable in livestock breeding programs (Bijma, 2000). The high expected rate of inbreeding in family selection programs is due to the fact that family information is the same for all family members and therefore their breeding values are very much alike as well. When animals would be selected based on their breeding values, this means that complete families would be selected. In the case of e.g. between family selection, 100 half-sib families of 100 fish are present, thus when 50 sires are selected, they would be all selected form the same family. When the number of parents is increased, family size consequently decreases and the selected sires would be from several families (Table 3).

When the rate of inbreeding was restricted to the rate of inbreeding achieved with mass selection (0.8%), the number of parents in the between family selection program had to go up by almost a factor 4 and a factor 3 in the combined within- between family selection program. When the numbers of parents were increased, the selection response from combined within-between family selection was comparable to mass selection (63.9g) whereas between family selection yielded lower selection response (53.5g). BLUP selection did not yield extra selection response compared to combined family selection. Probably the information content of the

Table 3. Selection response (ΔG) for body weight at 300 days of age and corresponding rate of inbreeding (ΔF) from different selection strategies. Either the number of parents was fixed* or the rate of inbreeding was fixed. Column 4 indicates the number of sires (N_S), -dams (N_D) and offspring (O) necessary to achieve the results at a fixed rate of inbreeding.

Selection strategy	Fixed number of parents ΔG (ΔF)	Fixed rate of inbreeding ΔG (ΔF)	N_S-N_D (O)
Mass selection	64.6g (0.8%)		50-100 (100)
Between family selection	70.8g (10.0%)	53.5g (0.8%)	190-380 (26)
Combined within- and between family selection	79.2g (5.0%)	63.9g (0.8%)	150-300 (32)
BLUP selection	79.5g (5.0%)	64.2g (0.8%)	150-300 (32)

* Selection response was calculated using 50 sires, 100 dams and a population size of 10000 fish. Genetic parameters for body weight at 300 days of age were σ^2_P=26000, h^2=0.20, c^2=0.03 (see: Chapter IV); calculations were performed using the computer program SelAction (Rutten et al., 2002).

index in combined family selection and thus the accuracy was high already and therefore BLUP did not improve the accuracy of the index much. However, when dynamic selection rules are applied to BLUP breeding values and average additive genetic relationships among parents, the response from selection can be optimized at a predetermined rate of inbreeding (Meuwissen, 1997). In a simulation study, Meuwissen (1997) showed that his methodology can increase selection response by 21 to 60% compared to BLUP selection due to increased selection differentials.

As indicated, mass selection offers a relatively cheap and simple strategy to realize breeding goals consisting of one trait. Even testing of large quantities of fish is much cheaper in mass selection, in terms of facilities and labor, compared to family- or BLUP selection. In Chapter IV, it was shown that the genetic correlation between fillet weight and body width was of such magnitude (0.94±0.06) that body width can be easily used as predictor for fillet weight of a fish. This means that large quantities of fish can be sorted based on body width to select the best with the aid of e.g. sorting machines. It is easy to imagine that very high selection intensities can be achieved in this way. Furthermore, the genetic correlation between body width and fillet yield was high (0.98±n.a.) which indicates that selection for fillet yield would even be possible when desired. Mass selection clearly offers very interesting perspectives for breeding programs in Nile tilapia, provided that traits can be measured on live animals that are genetically correlated to the traits of interest for genetic improvement.

The former discussion was led from the standpoint that the breeding goal contained only one trait. When the breeding goal is extended to contain more or other traits, the choice for the most efficient selection strategy could also change. The choice between e.g. family- and BLUP selection depends on the traits targeted for genetic improvement because both strategies have certain specific advantages. Family selection for instance, will allow measurements to be taken on complete families like e.g. feed conversion. In contrast, BLUP selection can yield more reliable breeding values in the mid- to long term due to build up of pedigree and also will allow periodical updating of genetic parameters. Nevertheless, breeding programs that are started using mass selection, can be easily extended to enable family selection or BLUP selection when desired. In that respect, investments into facilities enabling mass selection are not purposeless.

Extending the breeding goal

In this thesis, attention was mostly paid to production traits like body weight and fillet weight (Chapters III, IV, V and VI). These traits are directly related to a producer's income and are therefore important. In the long term, however, several traits can be thought of that are of economical importance for fish producers: e.g. feed conversion, disease resistance, amount of abdominal fat, fillet color, fertility traits or competition. Because of the fact that breeding programs in fish are still in a developing phase, it is easy to start off focusing on only one or two traits for genetic improvement. Later on, when the program is running, other traits can be incorporated in the breeding goal. The same development has occurred traditional livestock species: e.g. up until the 1990s, although a lot of traits where measured, the focus in dairy cattle breeding was mostly on production traits whereas at this moment traits like longevity, female fertility and resistance to mastitis are incorporated in selection indexes. Interestingly enough, attention has been drawn to some of these traits not because of direct economic interest, but because correlated responses from strong selection on production traits has led to undesired correlated responses in other traits. Examples can be found in e.g. physiological problems (ascites) in broilers, decreasing fertility in dairy cattle and decreasing intramuscular fat levels (meat quality) in pigs (for extended review see; Rauw et al., 1998). This indicates that it is essential to observe the population constantly so that signals of undesired correlated responses can be detected in an early stage and actions can be taken when necessary. At such a moment, several traits should be measured to investigate whether changes are truly taking place.

Feed conversion ratio

In intensive livestock production as well as in fish production, a large percentage of the production costs are feed costs (US figures: tilapia 34%, catfish 52%, broilers 60%; (Timmons, 2000). Genetic improvement of direct production traits results in e.g. faster growing animals in the case of selection for body weight. Effectively, more animals can be reared per time unit using the same facilities, and turnover is increasing. However, both Sanchez et al. (2001) and Mambrini et al. (2004) showed that feed conversion ratio did not change in selection experiments for growth in brown trout. This means that their genetically improved fish simply ate more feed to realize faster growth. According to Smith et al. (1988) and Thodesen et al. (1999) this is usually the case in fish when selection is on growth. Accessible research investigating whether feed conversion is heritable in fish is scarce for species like trout and

salmon, and non-existing for Nile tilapia. However, when the same relations between growth and feed-use exist in Nile tilapia, it is evident that incorporation of feed conversion ratio in the breeding goal could reduce feed costs (Gjedrem, 1983; Kinghorn, 1983; Bentsen, 1990; Gjerde et al., 2002) and increase production efficiency.

Measuring individual feed intake poses a problem in fish because it is not feasible in large scale breeding programs (Gjerde et al., 2002). In experiments this problem is usually overcome by measuring feed conversion in full-sib families (see: Kinghorn 1983; Henryon et al., 2002). Family selection, where facilities are required to accommodate many families for rearing, would enable selection for feed conversion. This means that more extensive investments are required compared to mass selection strategies, to enable selection for feed conversion in Nile tilapia. However, when the breeding goal is extended, feed conversion should be given priority over other traits because of its economical relevance.

Other indicators of efficiency

When family selection would be applied, a number of animals that are not selected could be sacrificed to obtain measurements not only of fillet weight, but also abdominal fat, fillet color and other characteristics that cannot be measured on live animals. It would be advisable in this case to choose animals within the whole range of body weights present, because otherwise traits correlated to body weight could be estimated inaccurately. Other ways to obtain measures of efficiency (fat content) in fish are available by computerized tomography (CT) scan (Rye, 1991), although this method seems to be too expensive for practical application at this moment (Gjerde, 2002).

A different approach to measures of efficiency can be given by examining differences in growth curves among fish. In Chapter V it was shown that genetic variation for the shape of growth curves exists in Nile tilapia. Whether these differences originate from differences in feed intake capacity or feed conversion is yet unknown. Generally, weight gain in animals shifts from protein gain to fat gain when life progresses and therefore, fast development of body weight early in life is often more efficient. Van der Werf (2002) developed methodology to alter the shape of a growth curve by selection. When information becomes available that connects some measure of efficiency with the shape of (partial) growth curves, it might be possible to optimize the efficiency of the complete growth curve for certain production circumstances.

In this Chapter, several aspects of selection strategies have been discussed. Earlier it was shown that mass selection offers excellent prospects for selection in Nile tilapia as long as the (correlated) traits of interest can be measured on live animals. When more complicated traits are desired, facilities can be expanded and e.g. family selection offers prospects to include measures of efficiency into the breeding goal. In Chapters III and IV it was shown that it is relatively hard to select for fillet yield and prospects for genetic improvement are moderate because of its relatively low genetic variation in Nile tilapia. Therefore, it is advisable to select for measures of weight that show relatively large genetic variation in Nile tilapia. When breeding goals are extended, measures of efficiency should be given priority because of their economic importance. In this case, extra investments would be required to enable other selection strategies because mass selection will not suffice anymore.

Variation

In many livestock species, variation is an important aspect for two reasons. On the one hand, variation in end-products is often undesirable, while on the other hand (genetic) variation is essential to be able to improve a population genetically. In this thesis, variation was dealt with in many instances. In Chapter II, it was shown that on the molecular level extended genetic variation is available within- and between randomly collected strains of Nile tilapia. When molecular genetic variation is found in a population, it is hypothesized that this is indicatory for the populations (statistical) variation in trait characteristics (Ritland, 1999). The presence of (genetic) variation for production traits was confirmed in Chapters III to V. In fact, relatively large (additive genetic) variance was found within Nile tilapia strains for several production traits. In the same Chapters (III to V), it was also shown that between sexes, large differences can be found in production traits of Nile tilapia where male fish are usually larger than female fish. Differences between sexes are not only undesirable for fish producers, but they also complicate selection procedures in fish breeding programs using mixed sex populations. Because distributions of body weight of male and female fish overlap largely, selecting the largest female fish is not straightforward. When large quantities of fish are involved in selection procedures, samples have to be drawn and sexed to determine the average weight and standard deviation of male and female fish so that it becomes clear in what weight range the best female

fish can be found. Provided that male and female fish would have similar average weights, large quantities of fish could be sorted by mechanical sorting machines and very high selection intensities and consequential selection response could be obtained.

Variation between sexes

In Nile tilapia, both within-sex- and between-sex variation of body weight is large: on average, male fish can weigh twice the body weight of female fish at mature age (Chapter V). Between-sex variation is common to numerous species of fish, mammals, birds etc. (see: Lande, 1980, and references herein). In Nile tilapia, the origin of this phenomenon has been attributed to e.g. behavioral factors (Schreiber et al., 1998), social interactions (Toguyeni et al., 2002), food deprivation in mouth-brooding female fish (Maar et al., 1966; Huet, 1972) and genetic factors (fryer and Iles, 1972). Schreiber et al. (1998) experimentally showed that female Nile tilapia grew faster than males when fish were reared individually. In recirculation systems, fish can be kept at high densities while guaranteeing constant water quality. With respect to Nile tilapia, high fish densities should prevent male fish to claim and defend territory, and prevent female fish from onset to ovulation. It has been hypothesized that in this way, energy is redirected from mating behavior and its physiological processes to investment in body weight. As a result of this, between-sex variation and consequently the total variation should diminish which is desired in the fillet processing industry. However, in Chapter V, it was clearly shown that average body weights of male and female fish were significantly different (α=0.05) already at ages around 122 days and at all higher ages. Therefore, the culture of Nile tilapia under high densities does clearly not lead to a reduction of the difference in average weight of male and female fish and the present variation.

Prospects for quantitative genetics to reduce the difference in average body weight between male and female fish were investigated in Chapter V. Genetic correlations between male and female body weights were estimated at various ages and were all high (0.99, 0.99, 0.99, 0.85 and 0.92 at the ages of 122, 151, 202, 234 and 293 days respectively). To be able to change the differences in average weight, a low genetic correlation would be required. Therefore, the results of this study suggest that selection probably has little impact to overcome sexual dimorphism in Nile tilapia.

Reduction of variation between sexes by use of monosex populations

The production and use of monosex populations to reduce the total variation in Nile tilapia, has received a lot of attention in research in the last decade. Under normal circumstances females carry the homogametic XX- and males the heterogametic XY sex-chromosome configuration in Nile tilapia. However, Nile tilapia has the ability to change its phenotypic sex, independently from its sex-chromosome configuration: masculinization occurs when larvae are exposed to high water-temperatures (Baroiller et al., 1995; Baras et al., 2001) and by using hormone-treated feed, both feminization and masculinization can be achieved (Shelton et al., 1978; Mair et al., 1987). In order to be effective, sex-reversal treatments have to be applied from the moment of first feeding up to approximately 30 days post-hatching. However, the underlying genetic mechanism of sex-reversal is still unknown and departures from the expected ratio of male to female offspring can still occur after sex-reversal treatment. Therefore, next to sex-linked genes, it is hypothesized that several (autosomal) loci carrying sex determining genes are involved in phenotypic sex determination (Karayücel et al., 2004).

The use of all-male populations has been advocated because these grow faster than mixed-sex populations and all-female populations (Mair et al., 1995). However, rather surprisingly, no information seems to be available with respect to feed efficiency and whether all-male populations are truly more efficient. All male populations can be produced by using YY male technology (Scott et al., 1989). The process goes as follows: By means of sex reversal, sex-reversed females (denoted by XY♀) are created and subsequently mated to normal males (XY). In their progeny, all sex-chromosomes will segregate according to Mendelian laws, which results in XX, XY and YY progeny according to the ratio 1:2:1. Without interference, animals carrying YY sex-chromosomes develop as normal males phenotypically. Differences between XY and YY males cannot be identified at the phenotypic level, therefore test matings with normal XX females are performed, and according to the sex-ratio of the produced progeny, YY males are identified (Mair et al., 1997). The identified YY males can subsequently be used in a multiplier step of a breeding program to produce all male progeny for rearing.

Several authors showed the feasibility of YY male production, but always at small scale. Mair et al. (1997) presented a breeding program structure where YY males can be produced at large scale. To accomplish this, several steps follow after the steps already described so that eventually also YY females are produced. When this is achieved, matings between YY♂ and

YY♀ always results in YY male progeny. In literature, no reports exist that YY-male technology has actually been incorporated within running Nile tilapia breeding programs.

Other possible scenarios can be thought of which use the ability of sex reversal in Nile tilapia to optimize efficiency of breeding programs. Earlier, the procedure to produce YY males was described. Actually, each phenotypic sex can be combined with each sex-chromosome configuration. Therefore, by using the same techniques, XX males can be produced (Toguyeni et al., 2002). In theory, this opens the perspective to develop a separate homogametic sire line (YY) and even homogametic dam lines (XX), where the focus for genetic improvement could be on different traits such as fertility in the female line and fillet weight in the sire line. Similar strategies are used in e.g. pig breeding (Smith, 1964). In theory, several advantages arise from this strategy: 1) sire- and dam line will diverge genetically with time, consequently, possible effects of heterosis could arise and be utilized; 2) since the end product is a cross of two different lines, the genetic characteristics of the parental lines are protected when only crossbreds are sold; 3) by using homogametic sex chromosome configurations in the nucleus, progeny bred for production of fillets will be all male (XY) with the benefit of higher total output compared to normal mixed sex progeny; and 4) in the nucleus, male and female animals would always have to be reared separately, which enables sorting of large quantities mechanically and results in high selection intensities. In each of the lines, homogametic progeny would be produced each generation that would develop only into female fish in the dam line (XX) and only into male fish in the sire line (YY) without interference. Therefore, some of the progeny in both lines would have to be sex reversed; otherwise future within-line matings would not be possible anymore. A major disadvantage of this method is the continuous use of hormones for sex-reversal, although masculinization could be achieved by an increase of water temperature (Baras et al., 2001). However, when consumers oppose against the use of hormones in the food chain, only small batches of sex-reversed animals could be produced, so that different selection intensities would be achieved in male and female fish within each line. Subsequently, only a number of sex reversed animals would have to be excluded from the food chain.

Reduction of variation by reducing effects of competition

In Chapter VI, it was shown that methodology exists to estimate genetic effects of competition. In this study, genetic effects of competition could not be identified in Nile tilapia although the

power of the design was not optimal. Genetic effects of competition open prospects to improve the population for effects of competition by BLUP- or group selection (i.e. where complete (randomly composed) groups are selected based on their average performance). The advantage of this method would be twofold: 1) the eventual production efficiency would be higher due to decreased losses in terms of survival and recovery from aggressive behavior, and; 2) due to less interactions between animals, the phenotypic differences would be smaller which would result in decreased phenotypic variance and a more homogeneous end-product.

Several aspects within the study in Chapter VI are still unclear at this moment. When e.g. animals die due to effects of competition during rearing, this is indicatory of the behavior of other animals in the same environment. At this moment, however, it is unclear what the best way is to include this information in the analysis. Furthermore, optimizations might be possible in the design of the experiment such as the population's family structure and the allocation of animals to communal environments. However, estimation of breeding values and selection on effects of competition using a similar model has already been proven effective by Muir and Schinckel (2002) in Japanese quail.

Further research into this methodology might even increase the ability to detect genetic effects of competition in the future. Because effects of competition and cannibalism are common in fish production, the presented methodology should find broad use. Although no genetic effects of competition could be detected in Nile tilapia (Chapter VI), the existence of these effects might not be ruled out and larger experiments should be designed to solve this issue.

Reduction of variation by (in)breeding strategies

In plant breeding, selection for homogeneous products has been established for a long time. The strategy there is to produce F1 hybrids from two inbred parental lines. The F1 progeny has little to no genetic variation, and as a result, increased uniformity is achieved, at least within the same environment. In animal breeding, the same strategy does not seem to work. Although it is easy to achieve high levels of genetic uniformity by repeated mating of relatives, and even easier in fish by use of androgenesis or gynogenesis (Komen, 1990), inbred lines of animals do not show reduced phenotypic variance compared to outbred populations. Why this happens is unclear. The heritability of a trait indicates by what amount the total variance could be reduced when the genetic variance is reduced to zero. In this thesis, heritabilities roughly around 0.2 were found for body weight in Nile tilapia. This means that a reduction of 20% of the total

variance would be possible when inbreeding strategies would be used. Since the total variance is relatively large for traits like body weight in fish, inbreeding strategies as used in plant do not seem promising in fish to produce a more homogeneous product.

Four methods were discussed to reduce the total variance in production traits of Nile tilapia. Selection to close the phenotypic difference in body weight between male and female fish can be ruled out because genetic correlations are too high. Using (in)breeding strategies, variance reductions approximately equal to the magnitude of the heritability could be achieved. However, it is probably complicated to design breeding programs where continuous genetic improvement could be realized in the nucleus and at the same time inbred animals for production are produced. Genetic variance for effects of competition could not be identified in this study. Larger experiments are needed first and when genetic variance of competition effects are identified in Nile tilapia, thereafter group- or BLUP selection could be implemented. Therefore, given the current knowledge in Nile tilapia, the use of monosex populations seems the only possible way to implement strategies to reduce variation in production of Nile tilapia.

Monitoring selection response in mass selection programs

In the introduction of this thesis, the steps involved in setting up a breeding program were discussed. One of these steps embodies the calculation of predicted selection response from the breeding program. After implementation of the breeding program, however, when a few generations have been produced, an important step is to monitor changes in the population's performance to be able to evaluate whether the breeding program is truly effective. Monitoring genetic improvement often posses a problem, because changes in the populations mean performance can be the consequence of many factors: genetic improvement, but also improved management, environmental- or seasonal fluctuations etc. Especially in mass selection programs, these effects are hard to disentangle, because no pedigree is available. The use of mixed model technology is, therefore, not possible and this means that special efforts are required to obtain an indication of the true effectiveness of the breeding program.

Hill (1972a, b) reviewed a number of methods to separate genetic- from environmental effects of which two could be used in mass selection programs. These methods are: 1) the use of an

unselected control population, or 2) the use of divergently selected lines. Both methods are based on regression of population performance on a time related variable of some form to level out yearly fluctuations. The contrast between the selected- and control or divergently selected groups subsequently give an indication of the genetic improvement of the selected population. However, both methods have requirements that are either hard to fulfill or costly, or are based on assumptions that are easily violated.

In the first method, an unselected control population is maintained next to the selected population. Random genetic drift can cause a genetic (level) change in the control population which needs to be accounted for. Furthermore, it is assumed that environmental effects are the same for both populations. In addition, the use of a control population causes production losses and takes away selection intensity from the breeding program and is therefore costly.

In the second method, next to the population selected in the desired direction, a population selected in the undesired direction is maintained. The production losses are larger per animal in the divergently selected group, but due to a higher contrast in performance to the selected group, the number of animals for testing could be reduced. In this method, equal influence of environmental factors for both populations is assumed.

Potentially, 4 sources of error might compromise the disentangling of environmental and genetic change using the described methods: 1) random genetic drift in the control population; 2) natural- or unintentional selection in the control population; 3) interaction between the environment and the genotypes of the control- and selected population, and; 4) error of estimation of the control population mean due to few individuals. The first three sources of error accumulate over generations but the last source of error does not and can be easily minimized by using sufficient animals (Hill, 1972a).

The use of both methods would require a part of the facilities and compromise selection response of the breeding program to some extend. Therefore, an alternative scheme proposed for use in pigs by the Meat and Livestock Commission (MLC; 1970) is presented here in adjusted form, for use in Nile tilapia breeding programs. The aim is to disentangle environmental- from genetic change and meanwhile minimize the costs of maintaining a control population and the subsequent loss in production. The MLC proposed to replace a control population every few generations by a new and improved control population and thereby reduce losses. In Nile tilapia this idea could be adopted by producing a control population every few generations from the selected population. The 1st adjustment to the MLC

scheme would be to use a control population for comparison to a generation of selected animals and discontinue the control population afterwards until a new control population is required. In Figure 1, a schematic overview is presented.

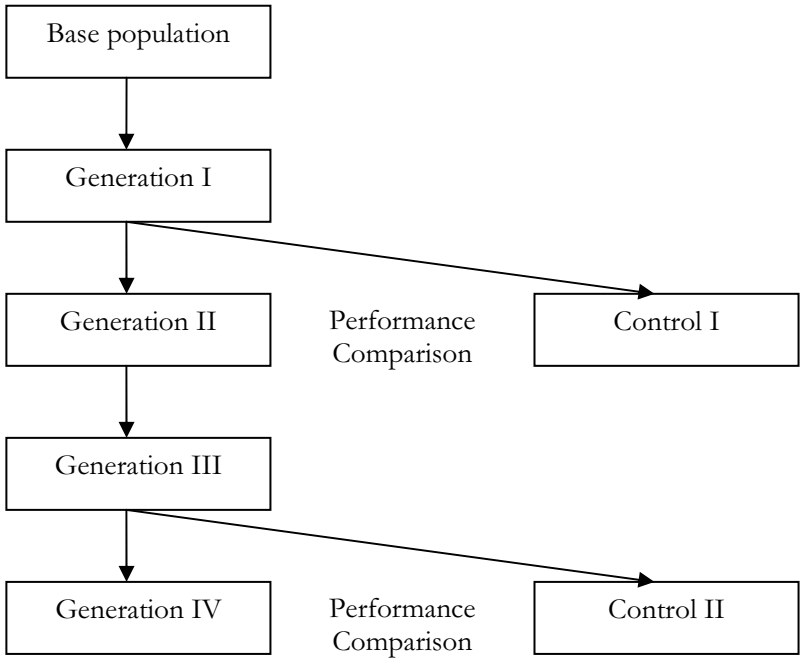

Figure 1. Schematic overview of the use of control populations in each 2nd generation in a mass selection program

In practice, progeny of selected parents would be tested against progeny of a number of randomly chosen parents, where it would be assumed that their average breeding value equals zero. The performance difference between these two groups would represent the amount of selection response of one generation.

Because of the fact that variation is relatively large in fish, the difference between the selection intensity of the selected parents and the randomly chosen parents can be very large. By rearing the control group instead of a selected group of fish, large production losses can be expected. Therefore, a second adjustment could be implemented in the MLC scheme to limit the production losses from using a control population. To achieve this, the parents of the control population should be chosen such that the contrast of their progeny's performance to the selected progeny's performance is large enough to obtain statistical power to detect a difference, but at the same time as small as possible to limit production losses. In this way, no correction for genetic drift is required, no unintentional- or natural selection takes place, and

due to the fact that both populations have diverged genetically by only 1 generation, effects of genotype by environment will be negligible. The accumulating sources of error as defined by Hill (1972a) can thus be ignored using this method.

In intensive aquaculture, usually large numbers of fish are reared in one tank. Therefore, high statistical precision can be achieved to detect a difference in performance of two groups. To determine the average breeding value of the parents of the control group, the following would have to be calculated: 1) The standard error of the performance difference of both groups could be calculated with the aid of the numbers of observations in each group and the standard deviation in each group (or the population's standard deviation when the actual standard deviations in each group were not available yet e.g. in the design phase). Using the standard error, a confidence interval for the difference can be calculated and an indication can be obtained on the minimum difference in performance that can be detected using these groups i.e. when the lower limit of the confidence interval is bigger than zero. 2) Based on the number of parents, the heritability and the phenotypic variance, the standard errors of both groups of parents can be calculated and a confidence interval can be constructed. Subsequently, the difference in average breeding values of the parents of both groups should be chosen such that the lower limit of the confidence interval of the difference in average breeding values is larger that the upper limit of the confidence interval of the performance difference. Since the average breeding value of the selected parents is known from the selection intensity, the average breeding value for the parents of the control group can be determined. Knowing the average breeding values of both groups of parents, the expected performance difference of the offspring can be determined. When the expected performance difference differs significantly from the realized performance difference, the population's genetic parameters might not be valid anymore and investigations into its causes would be in place.

Currently, methods that are not based on assumption that might be violated in practice are not present to monitor selection response in mass selection programs. The methodology presented here, assumes that tank effect are not present. In the data used in Chapter V, tanks effects were roughly around 5 grams at an average body weight of over 600 grams (not shown). This means that the effect of tank was less than 1%. However, when groups of 10000 fish are used and a phenotypic variance of 23231g^2 (Chapter V) is valid, differences in the range of approximately 5 grams can already be significantly detected. Therefore, the assumption that tank effects are

negligible seems justified but can now interfere with the testing of groups. To overcome this problem, it would be advisable to choose the parents of the control group such that the expected performance difference is somewhat larger than the effect of tank.

Although testing procedures for mass selection are not perfect, this should not withhold breeders to use one. The method proposed here can give an indication of the effectiveness of the breeding program at minimum costs so that losses are minimized. Griffing (1967) showed that selection response can even be in the undesired direction when e.g. interacting genotypes are present (Chapter VI). Therefore using for example the proposed method to get and indication whether a breeding program is effective, should always be preferred over no monitoring at all.

References

Baras, E., Jacobs, B., Melard, C., 2001. Effect of water temperature on survival, growth and phenotypic sex of mixed (XX-XY) progenies of Nile tilapia Oreochromis niloticus. Aquaculture 192, 187-199.

Baroiller, J.-F., Chourrout, D., Fostier, A., Jalabert, B., 1995. Temperature and sex-chromosomes govern the sex ratios of the mouthbrooding chiclid fish (*Oreochromis niloticus*). J. Exp. Zool. 273, 216-223.

Bentsen, H.B., 1990. Application of breeding and selection theory on farmed fish. Proc. 4th World Congr. Genet. Appl. Livest. Prod., Edinburgh, 23-27 Jul., vol. XVI, pp.149-158.

Bentsen H. B., Eknath A. E., Palada-de Vera M. S., Danting J. C., Bolivar H. L., Reyes R. A., Dionisio E. E., Longalong F. M., Circa A. V., Tayamen M. M. & Gjerde B. (1998) Genetic improvement of farmed tilapias: Growth performance in a complete diallel cross experiment with eight strains of *Oreochromis niloticus*. *Aquaculture* **160**, 145-73.

Bijma, P. (2000) Long-term genetic contributions. Prediction of rates of inbreeding and genetic gain in selected populations. PhD thesis, Animal Breeding and Genetics Group, Wageningen University, The Netherlands, pp 205-6

Bolivar R. B. & Newkirk G. F. (2000) Response to selection for body weight of Nile tilapia (Oreochromis niloticus) in different culture environments. In: *Proceedings from the Fifth International Symposium on Tilapia Aquaculture* **1**, 12-23.

Coward K. & Bromage N. R. (2000) Reproductive physiology of female tilapia broodstock. *Reviews in Fish Biology and Fisheries* **10**, 1-25.

Dey M. M. & Gupta M. V. (2000) Socioeconomics of disseminating genetically improved Nile tilapia in Asia: an introduction. *Aquaculture Economics and Management* **4**, 5-11.

Fessehaye, Y., Rezk, M., Bovenhuis, H., Komen, H., 2004. Size dependent cannibalism in juvenile tilapia (*Oreochromis niloticus*). Proceedings of the VIth international symposium on tilapia Aquaculture, Manila, Philippines, pp 230-238.

Fryer, G., Iles, T.D., 1972. The cichlid fishes of the great lakes of Africa: Their biology and distribution. Oliver and Boyd, Edinburgh.

Gall, G.A.E., Bakar, Y., 2002. Application of mixed-model techniques to fish breed improvement: analysis of breeding-value selection to increase 98-day body weight in tilapia. Aquaculture 212, 93-113.

Gjedrem, T., 1983. Genetic variation in quantitative traits and selective breeding in fish and shellfish. Aquaculture 33, 51-72.

Gjerde, B., Villanueva, B., Bentsen, H.B., 2002. Opportunities and challenges in designing sustainable fish breeding programs. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, August 19-23, Montpellier, France, Communication N° 06-01.

Griffing, B., 1967. Selection in reference to biological groups. IV. Application of selection index theory. Aust. J. Biol. Sci. 22, 131-142.

Hill, W.G., 1972a. Estimation of genetic change. I. General theory and design of control populations. Anim. Breed. Abstr. Vol. 40, No 1, 1-15.

Hill, W.G., 1972b. Estimation of genetic change. II. Experimental evaluation of control populations. Anim. Breed. Abstr. Vol. 40, No 2, 193-213.

Hecht, T., Pienaar, A.G., 1993. A review of cannibalism and its implications in fish larviculture. J. World Aquaculture Soc. 24, 246-261.

Henryon, M., Jokumsen, A., Berg, P., Lund, I., Pedersen, P.B., Olesen, N.J., Slierendrecht, W., 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture 209, 59-76.

Huet, M., 1972. Breeding and cultivation of fish. Fishing News (Books) Ltd., Farnham, Surrey.

Hulata, G., Wohlfarth, G., Halevy, A., 1986. Mass selection for growth rate in the Nile tilapia (Oreochromis niloticus). Aquaculture 57, 177-184.

Karayücel, I., Ezaz, T., Karayücel, S., McAndrew, B.J., Penman, D.J., 2004. Evidence for two unlinked "sex reversal" loci in the Nile tilapia, Oreochromis niloticus, and for linkage of one of these to the red body color gene. Aquaculture 234, 51-63.

Kinghorn, B.P., 1983. A review of quantitative genetics in fish breeding. Aquaculture 31, 283-304.

Komen, J., 1990. Clones of common carp. Doctoral Thesis, Agricultural University Wageningen, Wageningen, The Netherlands.

Lande, R., 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34, 292-305.

Maar, A., Mortimer, M.A.E., Van der Lingen, I., 1966. Fish Culture in Central East Africa. FAO, Rome, Italy.

Mair, G.C., Penman, D.J., Scott, A.G., Skibinski, D.O.F., Beardmore, J.A., 1987. Hormonal sex-reversal and the mechanism of sex determination in Oreochromis. In: Tiews, K. (Ed.), Proceedings of the World Symposium on Selection, Hybridization and Genetic Engineering in Aquaculture, vol. II. Heeneman, Berlin, pp. 301-311.

Mair, G.C., Abucay, J.S., Beardmore, J.A., Skibinski, D.O.F., 1995. Growth performance trials of genetically male tilapia (GMT) derived from YY-males in *Oreochromis niloticus* L.: On station comparisons with mixed sex and sex reversed male populations. Aquaculture 137, 313-322.

Mair, G.C., Abucay, J.S., Skibinski, D.O.F., Abella, T.A., Beardmore, J.A., 1997. Genetic manipulation of sex ratio for the large-scale production of all-male tilapia, Oreochromis niloticus. Can. J. Fish. Aquat. Sci. 54, 396-404.

Mambrini, M., Sanchez, M.P., Chevassus, B., Labbe, L., Quillet, E., Boujard, T., 2004. Selection for growth increases feed intake and affects feeding behavior of brown trout. Livest. Prod. Sci. 88, 85-98.

Martinez, V., Neira, R., Gall, G.A.E., 1999. Estimation of genetic parameters from pedigreed populations: lessons from analysis of alevin weight in Coho salmon (*Oncorhynchus kisutch*). Aquaculture 180, 223-236.

Meat and Livestock Commission, 1970. Pig improvement. Report of a scientific study group. London, UK.

Meuwissen, T.H.E., 1997. Maximizing the response of selection with a predefined rate of inbreeding. J. Anim. Sci. 75, 934-940.

Muir, W.M., Schinckel, A., 2002. Incorporation of competitive effects in breeding programs to improve productivity and animal well being. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, August 19-23, 2002, Montpellier, France. Communication N° 14-07.

Pantastico, J.B., Dangilan, M.M.A., Eguia, R.V., 1988. Cannibalism among different sizes of tilapia (*Oreochromis niloticus*) fry/fingerlings and the effect of natural food. Proceedings of the Second International Symposium on Tilapia in Aquaculture, 465-468.

Pante, M.J.R., Gjerde, B., McMillan, I., Misztal, I., 2002. Estimation of additive and dominance genetic variances for body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 204, 383-392.

Rauw, W.M., Kanis, E., Noordhuizen-Stassen, E.N., Grommers, F.J., 1998. Undesirable side effects of selection for high production efficiency in farm animals: a review. Livest. Prod. Sci. 56, 15-33.

Ritland, K., 2000. Marker-inferred relatedness as a tool for detecting heritability in nature. Mol. Ecol. 9, 1195-1204.

Rutten, M.J.M., Bijma, P., Woolliams, J.A., Arendonk, J.A.M. van, 2002. SelAction: Software to predict selection response and rate of inbreeding in livestock breeding programs. J. Hered. 93, 456-458.

Rye,M., 1991. Prediction of carcass composition in Atlantic salmon by computerized tomography. Aquaculture 99, 35-48.

Sanchez, M.P., Chevassus, B., Labbe, L., Quillet, E., Mambrini, M., 2004. Selection for growth of brown trout (*Salmo trutta*) affects feed intake but not feed efficiency. Aquat. Living Resour. 14, 41-48.

Schreiber, S., Focken, U., Becker, K., 1998. Individually reared female Nile tilapia (*Oreochromis niloticus*) can grow faster than males. J. Appl. Icht. 14, 43-47.

Scott, A.G., Penman, D.J., Beardmore, J.A., Skibinski, D.O.F., 1989. The 'YY' supermale in *Oreochromis niloticus* (L.) and its potential in aquaculture. Aquaculture 78, 237-251.

Shelton, W.L., Hopkins, K.D., Jensen, G.L., 1978. Use of hormones to produce monosex tilapia for aquaculture. In: Smitherman, R.O., Shelton, W.L., Grover, J.H. (Eds.), symposium on the culture of exotic fishes, fish culture section. American Fisheries Society, Bethesda, USA, pp. 10-33.

Smith, C., 1964. The use of specialized sire and dam lines in selection for meat production. Anim. Prod. 6, 337-344.

Smith, C., Reay, P., 1991. Cannibalism in teleost fish. Rev. Fish Biol. Fisheries 1, 41-64.

Smith, R.R., Kincaid, H.L., Regenstein, J.M., Rumsey, G.L., 1988. Growth, carcass composition, and taste of rainbow trout of different strains fed diets containing primarily plant or animal protein. Aquaculture 70, 309-321.

Sonesson, A.K., Gjerde, B., Meuwissen, T.H.E., 2005. Truncation selection for BLUP-EBV and phenotypic values in fish breeding schemes. Aquaculture 243, 61-68.

Tave, D., Smitherman, R.O., 1980. Predicted response to selection for early growth in tilapia Nilotica. Trans. Am. Fish. Soc. 109, 439-445.

Teichert-Coddington, D.R., Smitherman, R.O., 1988. Lack of response by tilapia Nilotica to mass selection for rapid early growth. Trans. Am. Fish. Soc. 117, 297-300.

Thodesen, J., Grisdale-Helland, B., Helland, S.J., Gjerde, B., 1999. Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (*Salmo salar*). Aquaculture 180, 237-246.

Timmons, B., 2000. Comparison of aquaculture and broiler production systems. AES News, Winter & Spring 2000, Vol. 3, Nos. 1 & 2, pp 8-11.

Toguyeni, A., Fauconneau, B., Fostier, A., Abucay, J., Mair, G., Baroiller, J.-F., 2002. Influence of sexual phenotype and genotype, and sex ratio on growth performances in tilapia *Oreochromis niloticus*. Aquaculture 207, 249-261.

Uraiwan, S., Doyle, R.W., 1986. Replicate variance and the choice of selection procedures for tilapia (Oreochromis niloticus) stock improvement in Thailand. Aquaculture 57, 93-98.

Villanueva, B., Wray, N.R., Thompson, R., 1993. Prediction of asymptotic rates of response from selection on multiple traits using univariate and multivariate best linear unbiased predictors. Anim. Prod. 57, 1-13.

Villanueva, B., Woolliams, J.A., Gjerde, B., 1996. Optimum designs for breeding programmes under mass selection with application in fish breeding. Anim. Sci. 63, 563-576.

Werf, J.H.J. van der, 2002. Optimising selection for traits along trajectories. Proc 7th WCGALP, Communication N° 16-07.

Wray, N.R., Hill, W.G., 1989. Asymptotic rates of response from index selection. Anim. Prod. 49, 217-227.

Summary

Nile tilapia has been the fish species with the largest production expansion in recent years. At the same time, interest in genetic improvement of Nile tilapia has increased because it can contribute to the improvement of production efficiency. Currently, the European market asks for fillets between approximately 100g and 150g because these are considered sufficiently large to serve as a meal for one person. Fillet yield of Nile tilapia varies roughly from 26% to 37% and therefore, fish need to weigh roughly between 700g and 800g. To develop a breeding program to improve mature body weight, genetic parameters for the design and optimization are indispensable. However, genetic parameters for body weight between 700 and 800g are scarce in literature. Selection for fillet yield in fish raises an additional problem: accurate phenotypic selection criterions for fillet yield in fish lack. When e.g. mass selection programs are targeted, measuring of fillet yield would mean that the animals are sacrificed and that they cannot be used for reproduction anymore. Therefore, the aim of this thesis was to generate knowledge that supports the design of breeding programs for Nile tilapia targeting genetic improvement of body weight and fillet yield to serve the European market.

To enable successful breeding programs, a population is required that shows an acceptable performance level and sufficient genetic variation for the traits of interest. To test both the genetic variation and the performance level of different strains of fish within intensive recirculation systems, two studies were performed: 1) Four domesticated strains of Nile tilapia (*Oreochromis niloticus L.*) were collected and genetically characterized using 14 microsatellite markers, in **Chapter II**. The average numbers of alleles per marker were 5.0 (GÖTT), 5.4 (AIT), 5.6 (IDRC) and 7.5 (GIFT). Private alleles were found at all markers with the exception of two. No fixation of alleles was found at any marker. Population differentiation, F_{ST}, was 0.178 (great genetic differentiation) and confirmed grouping of the animals in strains. The expected level of heterozygosity ranged from 0.624 to 0.711, but the observed level of heterozygosity significantly deviated from the expected level in three strains. Moderate to great genetic differentiation was found between strains. A phylogenetic tree reflected the strains known histories. Application of the Weitzman approach showed that all strains had added value for the total genetic diversity and thus should be retained. 2) Performance differences

with respect to fillet weight and fillet yield of Nile tilapia strains were investigated in **Chapter III**. In this study, slaughter data were collected on 1215 tilapia and used to analyze the relationship between body measurements and fillet weight and fillet yield. Average fillet yield was 35.7% with large differences between strains (range 34.4 –38%). There was a strong almost linear relationship between body measurements and fillet weight, but relationships with fillet yield were weak. Prediction models were developed for fillet weight and fillet yield. R^2 of the prediction model for fillet weight was 0.95 and the correlation between observed and predicted values of fillet weight 0.98. The effect of strain/origin was significant for each body measurement. The fillet yield model explained 15% of the observed variance; the correlation between observed and predicted fillet yield was 0.38, but there were large differences within strains. It was concluded that in Nile tilapia, predicting fillet yield based on body measurements is possible, but correlations can be improved if more accurate methods for measuring body width are available.

To be able to improve economically important traits, it is required to know to what extend these are heritable and what amount of genetic variation is available. Therefore, genetic parameters for body weight, fillet weight, fillet yield and body measurements were estimated in **Chapter IV**. Heritabilities were 0.26 for body weight, 0.24 for fillet weight and 0.12 for fillet yield. The genetic correlation between body weight and fillet weight was 0.99 and between body weight and fillet yield 0.74. The genetic correlation between fillet weight and fillet yield was 0.81. Genetic correlations between fillet weight and body measurements were generally high (0.70-0.94) and between fillet yield and body measurements fractionally lower (0.47-0.98). Using these genetic parameters, the potential of mass selection for fillet traits was evaluated. The accuracy of a selection index including only body weight indicated that in this way almost the same amount of the selection response can be achieved compared to what hypothetical direct selection for fillet weight would. The use of only width in the selection index would result in 8.5% lower selection response than the use of body weight. We concluded that body weight is the best predictor for fillet weight compared to body measurements.

Genetic parameters for body weight at ages over approximately 120 days are scarce in Nile tilapia. In **Chapter V**, genetic parameters for body weight in Nile tilapia were estimated for all ages ranging from 100 days to 326 days. Five repeated observations of body weight were analyzed using a random regression model with covariance functions. The heritability of body weight was fairly constant around 0.2, which offers good prospects for selection on body

weight. Genetic correlations were estimated between all ages (100-326 days). These genetic correlations enabled evaluation of the potential of early selection for body weight and showed that early selection results in higher selection response than direct selection, when the target trait is body weight at the age of 326 days. This was due to somewhat higher heritabilities at lower age and a shortened generation interval. Furthermore, evidence was found for genetic differences in growth patterns of fish of different strains. This means that the possibility to change the shape of body weight curves by selection exists and that the choice of strains should depend on the target market weight of the production chain. From the raw data we concluded that differences in body weight between male- and female fish were significant already at early ages (100 days). Results from a bivariate genetic analysis, where body weight of male- and female fish are treated as separate traits, suggest that body weight in male and female fish is most likely controlled by the same genes since genetic correlations were high (>0.85) at all five measurement dates. Prospects to decrease the difference between mature male- and female body weight by selection, to reduce the total variation, are therefore unfavorable in Nile tilapia.

In Nile tilapia-culture and in fish-culture in general, effects of competition can be commonly observed. Debate whether selection for growth rate increases competitive behavior in fish has been going on for decades. All this time, the theoretical framework to answer this question was available, but never recognized. New, methodology to estimate variance components for competitive genetic effects was applied to Nile tilapia in **Chapter VI**. No genetic variation for competitive behavior was found although environmental variation for competitive behavior could clearly be detected. This methodology is potentially very interesting for breeding in aquaculture, because no observations on behavior are required.

Throughout this thesis, studies were performed from the standpoint that mass selection should be used as selection strategy in Nile tilapia. In the General discussion in **Chapter VII**, the advantages and disadvantages of three selection strategies are discussed in terms of necessary facilities, workload and selection responses. It was shown that when traits measurable on live animals are targeted for genetic improvement, mass selection offers a cheap and simple strategy that can result in relatively high selection responses. However, when more complicated traits are desired, both family- and BLUP selection offer better possibilities depending on the trait of choice.

In this thesis, it was shown that body weight variation is relatively large in Nile tilapia. Four ways to try to diminish the phenotypic variation, so that a more uniform product can be

offered to the fish processing industry, were subsequently discussed. Selective breeding to improve either male- or female body weight offers no prospects because of the large genetic correlations found. Using (in)breeding strategies as is common in plant breeding to produce a uniform product, does not seem to work in animals. Therefore, using this strategy, a variance reduction, the size of the heritability, can be achieved at the most. Reduction of variation in Nile tilapia by reducing effects of competition does not seem to offer possibilities because no genetic variation for this trait seems to be present. The fourth method, the use of monosex populations, seems the only way to diminish variation of fish weight at this moment. Actually, very interesting scenarios can be thought of, of which one example is discussed.

The last topic that was discussed in **Chapter VII** is the need to monitor selection response in mass selection programs. Because no pedigree is available in mass selection programs, it is hard to disentangle genetic- from environmental trend or -fluctuations. The currently available methods depend on assumptions that are easily violated in practice. In addition, these methods are often costly, because they require additional groups of animals to be reared. Therefore a method is proposed to reduce the costs of rearing extra animals which can give an indication whether the breeding program is truly effective.

Samenvatting

Nijl tilapia is de vissoort met de grootste productie uitbreiding van de afgelopen jaren. Tegelijkertijd is de interesse voor fokprogramma's voor Nijl tilapia toegenomen, omdat deze kunnen bijdragen aan de verbetering van de productie-efficiëntie. Op dit moment vraagt de Europese markt filets tussen ongeveer 100g en 150g omdat deze als voldoende groot beschouwd worden om als maaltijd voor één persoon te dienen. Het filetpercentage van Nijl tilapia varieert ruwweg van 26% tot 37% en daarom moeten vissen dus tussen ruwweg 700g en 800g wegen. Om fokprogramma's te kunnen ontwikkelen die het volwassen lichaamsgewicht verbeteren, zijn genetische parameters voor de opzet en optimalisatie noodzakelijk. Echter, genetische parameters voor gewichten tussen 700g en 800g zijn schaars in de literatuur. Selectie voor filetpercentage geeft ook nog andere problemen: betrouwbare selectie criteria meetbaar aan het levende dier zijn niet voorhanden. Als b.v. massa selectie programma's beoogd worden, zou het meten van het filetpercentage betekenen dat dieren opgeofferd moeten worden en niet meer gebruikt kunnen worden voor reproductie. Het doel van dit proefschrift was daarom het genereren van kennis die de ontwikkeling van fokprogramma's ondersteunt voor de genetische verbetering van lichaamsgewicht en filetpercentage om de Europese markt bedienen.

Om succesvolle fokprogramma's mogelijk te maken, is een populatie noodzakelijk die voldoende genetische variatie bezit voor de beoogde kenmerken voor verbetering. Om zowel de genetische variatie als het prestatie niveau van verschillende lijnen vissen in intensieve recirculatie systemen te testen, zijn twee studies uitgevoerd: 1) In **Hoofdstuk II** zijn vier gedomesticeerde Nijl tilapia lijnen genetisch gekarakteriseerd met behulp van 14 microsateliet merkers. Het gemiddelde aantal allelen per merker was 5.0 (GÖTT), 5.4 (AIT), 5.6 (IDRC) and 7.5 (GIFT). Private allelen zijn gevonden op iedere merker met uitzondering van twee. Geen enkele merker was gefixeerd voor één allel. De populatie differentiatie, F_{ST}, was 0.178 (dwz grote genetische differentiatie) en bevestigde groepering van de dieren in verschillende lijnen. Het verwachtte niveau van heterozygotie varieerde van 0.624 tot 0.711, maar in drie lijnen week het geobserveerde niveau van heterozygotie significant af van het verwachtte niveau. De genetische differentiatie tussen lijnen die werd gevonden was gemiddeld tot groot. Een phylogenetische boom weergaf wat bekend was over de achtergronden van de lijnen.

Toepassing van de Weitzman methode gaf aan alle lijnen toegevoegde waarde hadden voor de totale genetische diversiteit en dus behouden zouden moeten worden. 2) In **Hoofdstuk III** zijn prestatieverschillen met betrekking tot filetgewicht en filetpercentage van Nijl tilapia lijnen onderzocht. Tijdens deze studie werd slachtdata verzameld van 1215 tilapia's en vervolgens gebruikt om de relatie tussen lichaamsmaten, filetgewicht en filetpercentage te analyseren. Het gemiddelde filetpercentage was 35.7%, maar tussen lijnen werden grote verschillen gevonden (van 34.4 – 38%). Er was een sterke, bijna lineaire, relatie tussen lichaamsmaten en filetgewicht, maar relaties met het filetpercentage waren zwak. Voorspellingsmodellen werden ontwikkeld voor filetgewicht en filetpercentage. R^2 van het voorspellingsmodel voor filetgewicht was 0.95 en de correlatie tussen de geobserveerde en voorspelde waarden van filetgewicht was 0.98. Het effect van lijn/achtergrond was significant voor alle lichaamsmaten. Het voorspellingsmodel voor filetpercentage verklaarde 15% van de geobserveerde variantie; de correlatie tussen geobserveerde en voorspelde waarden was 0.38, maar er waren grote verschillen tussen lijnen. De conclusie van deze studie was dat het voorspellen van filetpercentage in Nijl tilapia gebaseerd op lichaamsmaten mogelijk is, maar dat de correlaties verbeterd kunnen worden als er meer betrouwbare methodes voor het meten van lichaamsbreedte beschikbaar zijn.

Om het mogelijk te maken economisch belangrijke kenmerken te verbeteren, is het noodzakelijk te weten in welke mate deze erfelijk bepaald zijn. Daarom zijn in **Hoofdstuk IV** genetische parameters geschat voor lichaamsgewicht, filetgewicht, filetpercentage en lichaamsmaten. Erfelijkheidsgraden waren: 0.26 voor lichaamsgewicht, 0.24 voor filetgewicht en 0.12 voor filetpercentage. De genetische correlatie tussen lichaamsgewicht en filetgewicht was 0.99 en tussen lichaamsgewicht en filetpercentage 0.74. De genetische correlatie tussen filetgewicht en filetpercentage 0.81. De genetische correlaties tussen filetgewicht en lichaamsmaten waren over het algemeen hoog (0.70-0.94) en tussen filetpercentage en lichaamsmaten iets lager (0.47-0.98). Met behulp van deze genetische parameters is vervolgens het potentieel van massa selectie voor filetkenmerken geëvalueerd. De nauwkeurigheid van een selectie-index welke alleen lichaamsgewicht bevatte gaf aan dat op deze manier bijna dezelfde hoeveelheid selectie respons bereikt kan worden vergeleken met hypothetische directe selectie op filetgewicht. Het gebruik van alleen lichaamsbreedte in de selectie-index resulteerde in een ongeveer 8.5% lagere respons vergeleken met het gebruik van alleen lichaamsgewicht. We concludeerden dat lichaamsgewicht de beste voorspeller voor filetgewicht is vergeleken met lichaamsmaten.

Genetische parameters voor lichaamsgewicht op leeftijden groter dan ongeveer 120 dagen zijn schaars voor Nijl tilapia. In **Hoofdstuk V** zijn genetische parameters voor lichaamsgewicht in Nijl tilapia geschat voor alle leeftijden van 100 tot 326 dagen. Vijf herhaalde waarnemingen van lichaamsgewicht zijn geanalyseerd met een random regression model met covariantie functies. De erfelijkheidsgraad van lichaamsgewicht was redelijk constant rond 0.2, wat goede perspectieven voor selectie op lichaamsgewicht biedt. Genetische correlaties zijn geschat tussen alle leeftijden (100-326 dagen). De geschatte genetische correlaties maakten het mogelijk om het potentieel van vroege selectie op lichaamsgewicht te evalueren en dit gaf aan dat vroege selectie een hogere selectie respons kan geven dan directe selectie, als het beoogde kenmerk lichaamsgewicht op 326 dagen is. Dit werd veroorzaakt door iets hogere erfelijkheidsgraden op jongere leeftijd en een verkort generatie interval. Verder is er bewijs gevonden dat er genetische verschillen bestaan tussen groeipatronen van vissen van verschillende genetische lijnen. Dit betekent dat er een mogelijkheid bestaat om de vorm van een groeipatroon aan te passen met behulp van selectie en dat de keuze voor bepaalde lijnen moet afhangen van het beoogde aflevergewicht van de vis in de productieketen. Uit analyses van de ruwe data werd geconcludeerd dat verschillen in lichaamsgewicht tussen mannelijke en vrouwelijke dieren al op jonge leeftijd (100 dagen) significant waren. De resultaten van een bivariate analyse, waarin de lichaamsgewichten van mannelijke en vrouwelijke dieren als aparte kenmerken beschouwd werden, gaven aan dat lichaamsgewicht in Nijl tilapia hoogstwaarschijnlijk bepaald wordt door dezelfde genen omdat de correlaties hoog waren (>0.85) op alle vijf de meetmomenten. De vooruitzichten om de verschillen tussen mannelijke en vrouwelijke dieren te verkleinen met selectie, zodat de totale variatie ook verkleind word, lijken daarom niet gunstig.

In Nijl tilapia teelt en in visteelt in het algemeen, kunnen effecten van competitie regelmatig waargenomen worden. De discussie of selectie op lichaamsgewicht competitief gedrag verstekt is al decennia aan de gang. Gedurende deze tijd was het theoretische raamwerk om deze vraag te beantwoorden aanwezig, maar is nooit als zodanig herkend. Nieuwe methodologie om variantie componenten voor genetische effecten van competitie te schatten is toegepast op Nijl tilapia in **Hoofdstuk VI**. Er is geen genetische variatie voor competitief gedrag gevonden hoewel milieuvariatie voor competitief gedrag duidelijk aangetoond kon worden. Deze methodologie kan in potentie erg interessant zijn voor de fokkerij in de aquacultuur omdat er geen observaties aan gedrag nodig zijn.

In dit proefschrift zijn studies verricht met als uitgangspunt dat massa selectie gebruikt zou worden als selectie strategie in Nijl tilapia. In de Algemene discussie in **Hoofdstuk VII**, worden de voordelen en nadelen van drie selectiestrategieën besproken in termen van noodzakelijke faciliteiten, werklast en selectie respons. Er wordt getoond dat als kenmerken, meetbaar aan levende dieren, beoogd worden voor genetische verbetering, massa selectie een goedkope en simpele strategie biedt die kan resulteren in een relatief hoge selectie respons. Echter, als meer gecompliceerde kenmerken beoogd worden voor genetische verbetering, bieden zowel familie als BLUP selectie, afhankelijk van het gekozen kenmerk, betere mogelijkheden.

In dit proefschrift is getoond dat variatie in lichaamsgewicht relatief groot is in Nijl tilapia. Vier manieren om phenotypische variatie te verkleinen, zodat een meer uniform product aangeboden kan worden aan de verwerkende industrie, zijn vervolgens besproken. Selectie om ofwel het gemiddelde gewicht van mannelijke dieren ofwel het gemiddelde gewicht van vrouwelijke dieren te verbeteren biedt geen perspectief omdat hoge genetische correlaties gevonden zijn. Het gebruik van inteelt strategieën zoals gebruikelijk is in de plantenteelt om uniforme producten te produceren, lijkt niet te werken bij dieren. Daarom kan bij het gebruik van deze strategie ten hoogste een variantie reductie ter grootte van de erfelijkheidgraad bereikt worden. De reductie van variatie in Nijl tilapia door middel van het reduceren van de effecten van competitie lijkt geen mogelijkheden te bieden omdat er geen genetische variatie voor dit kenmerk aanwezig lijkt te zijn. De vierde methode, het gebruik van mono-sexe populaties, lijkt de enige manier om de variantie van lichaamsgewicht te reduceren op dit moment. Zeer interessante scenario's kunnen bedacht worden, waarvan er een besproken is.

Het laatste onderwerp dat besproken word in **Hoofdstuk VII**, is de noodzaak om selectie respons te monitoren in massa selectie programma's. Omdat er geen afstamming beschikbaar is in massa selectie programma's is het moeilijk om genetische trends van milieu trends of -fluctuaties te scheiden. De op dit moment beschikbare methodes zijn gebaseerd op aannames die gemakkelijk geschonden worden in de praktijk. Daarbij komt nog dat deze methodes vaak duur zijn omdat er extra groepen dieren gehouden moeten worden. Daarom is een methode voorgesteld om de kosten te reduceren die het houden van extra dieren met zich meebrengt en die een indicatie kan geven of het fokprogramma werkelijk effectief is.

Nawoord

En toen was het af!
Toen ik begon aan dit project werd er aan mijn onderzoekscapaciteiten niet getwijfeld, maar of ik me realiseerde dat ik af en toe ook "met de handen in het water" moest? Achteraf was dat lichtelijk understated! Tot aan m'n knieën komt meer in de richting, maar ik heb het met plezier gedaan.
Normaal gesproken staat in het nawoord van een proefschrift dat de promovendus het werk niet alleen heeft gedaan. In mijn geval zou dat weer een behoorlijk understatement zijn. Het waren er namelijk velen! Volledigheidshalve ga ik dus een heleboel mensen noemen.
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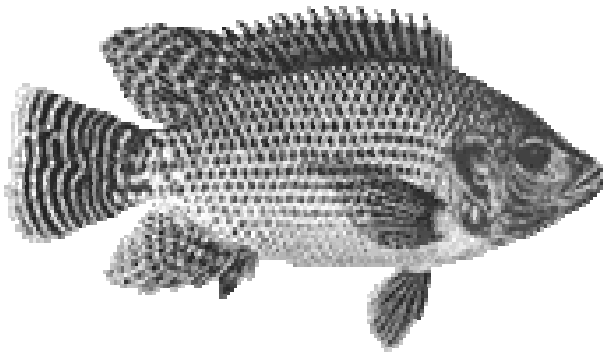
Curriculum vitae

Marc Joseph Maria Rutten werd op zondag 8 april 1973 geboren te Groesbeek. Na het lager onderwijs in Groesbeek doorlopen te hebben, behaalde hij in 1990 het HAVO diploma aan de Nijmeegse Scholengemeenschap Groenewoud. In datzelfde jaar begon hij met de studie veehouderij aan de toenmalige Agrarische Hogeschool 's-Hertogenbosch. Stage en afstudeervak werden uitgevoerd bij respectievelijk het toenmalige Zuid-Oost Genetics te Harfsen en het Nederlands Rundveesyndicaat (NRS) te Arnhem. In 1997 behaalde hij het ingenieursexamen. Nog in datzelfde jaar begon hij met de studie Zoötechniek aan de toenmalige Landbouw Universiteit te Wageningen, met als specialisatie Fokkerij en Genetica. Voor het eerste afstudeervak deed hij onderzoek naar de gevoeligheid van QTL-detectie methoden voor het verondersteld onderliggend genetisch model. Met een studiebeurs van de Prof. Rommert D. Politiek stichting heeft hij een tweede afstudeervak uitgevoerd bij het Centre for Genetic Improvement of Livestock (CGIL) van de University of Guelph te Canada. Voor dit afstudeervak werd onderzoek gedaan naar de implicaties van het gebruik van een test-dag model in een genetische evaluatie, onder begeleiding van prof. L.R. Schaeffer. In 1999 studeerde hij *cum laude* af, waarna hij tijdelijk aangesteld werd bij de leerstoelgroep Fokkerij en Genetica als programmeur van het software programma "SelAction", wat genetische vooruitgang en inteelt van fokprogramma's voorspelt. Vanaf december 2000 was hij als assistent in opleiding (AIO) aangesteld bij de leerstoelgroepen Visteelt en Visserij en Fokkerij en Genetica op het in dit proefschrift beschreven onderzoek. Vanaf januari 2005 is hij tijdelijk aangesteld als onderzoeker in een samenwerkingsverband tussen de leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit en Hybro te Boxmeer.

Oreochromis niloticus Linnaeus 1758



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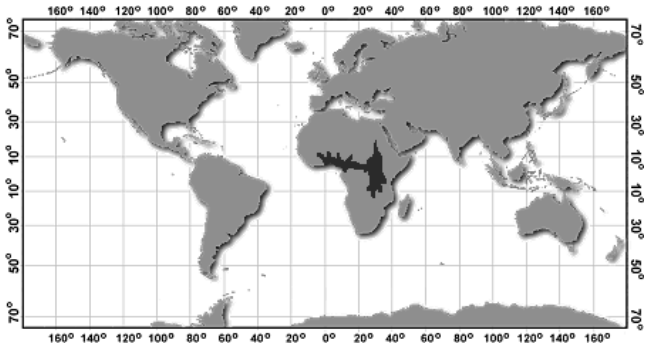

Sources: http://www.fao.org/figis/; http://www.fishbase.org/

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