Natural mating in Nile tilapia (*Oreochromis niloticus* L.)
Implications for reproductive success, inbreeding and cannibalism

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Dit onderzoek is uitgevoerd binnen de onderzoekschool WIAS

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Proefschrift
Ter verkrijging van de graad van doctor
Op gezag van rector magnificus
Van Wageningen Universiteit,
Prof. Dr. M.J. Kropff,
In het openbaar te verdedigen
Op maandag 11 december 2006
Des namiddags te half twee in de Aula.

Fessehaye, Y.

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PhD thesis, Wageningen University, 2006

With summary in English and Dutch

ISBN: 90-8504-540-1

To my parents:

Fessehaye and Ghidey Ghebreyesus

Contents

Chapter 1

General Introduction

1. Tilapia in aquaculture

Tilapia is the third most cultured fish, after carps and salmonids. In the last decade, the production of farmed tilapia has shown a tremendous increase jumping from 383,654 metric tons in 1990 to 1,505,804 metric tons in 2002. The value of farmed tilapia has also witnessed a great increase during the past two decades, going from US$154 million in 1984 to US$1800.7 million in 2002 (El-Sayed, 2006). About 16 tilapiine species have been used for aquaculture production out of which ten species are commercially farmed (FAO, 2004). Since 1984, global tilapia production is dominated by three species: the Nile tilapia *Oreochromis niloticus* (L.), the Mozambique tilapia *Oreochromis mossambicus* (Peters) and the blue tilapia *Oreochromis aureus* (Steindachner) (Rana, 1997). Today Nile tilapia, by far, is the most important farmed tilapia species, representing more than 80% of total tilapia production.

2. Selective breeding in Nile tilapia

Both small-scale and commercial production of tilapia is rapidly expanding in many countries. While remarkable progress has been made in improving the productivity of crops and livestock in the last three to four decades through breeding and selection, it is only in the last few decades that efforts have been made to harness the benefits of genetic improvement in fish. In Nile tilapia, different selection strategies such as mass selection (Tave and Smitherman, 1980; Hulata et al., 1986; Teichert-Coddington and Smitherman, 1988), within family selection (Bolivar and Newkirk, 2002) and combined within- and between family selection (e.g. ‘Genetic Improvement of Farmed Tilapias’ (GIFT) project: Eknath et al., 1993) have been used to improve growth. In the GIFT breeding programme, selective breeding in five consecutive generations resulted in a cumulative genetic improvement of 85% compared to the base population (Dey and Gupta, 2000). Most other breeding programmes were merely used to generate few generations of selection response and to estimate genetic parameters. As a result only few recommendations have been made with respect to the most efficient selection strategy for Nile tilapia. Gall and Bakar (2002) argued that BLUP (Best Linear Unbiased Prediction) selection is the most efficient method for selection in tilapia. However, Sonesson et al. (2005) showed that BLUP selection might result in unacceptably high rates of inbreeding and recommended mass selection as a more desirable strategy for fish breeding programmes.

Mass selection is the simplest form of selection within a population involving rearing large groups of animals simultaneously and then selecting the best, based on their phenotypic values (Falconer and Mackay, 1996). In fish, mass selection is often combined with mass spawning, where broodstock are mated ‘en masse’ in spawning enclosures. It has been a common strategy in fish breeding because of its simplicity and because it does not require individual identification or maintenance of pedigree records. In principle, mass selection can produce rapid improvement if the heritability of the traits under selection is high. However, earlier work on mass selection in tilapia showed no or marginal response to genetic improvement (Hulata et al., 1986; Huang and Liao, 1990). This disappointing response to mass selection in Nile tilapia has been attributed to the availability of low genetic variation in the base population and accumulation of inbreeding, non-heritable phenotypic variances (e.g. cannibalism) and wrong or inappropriate management practices. Due to high fecundity in many fish species, large numbers of offspring are produced in each generation while only few animals are actually selected for the production of a new generation, i.e. a very small number of individuals can make a large contribution to the genetic make-up of successive generations. This will lead to the fast accumulation of inbreeding and loss of additive genetic variance counteracting further genetic improvement (Gjerde and Rye, 1998). Moreover in mass spawning the contribution of parents is not known and genetic contributions might be dominated by few parents; exacerbating the accumulation of inbreeding. Inbreeding becomes more serious in small facilities with limited capabilities to maintain parent broodstock in large numbers.

In practicing mass selection, animals which display a better than average phenotype for a certain trait, are selected to produce future generations. However, mass-spawned fry consist of groups of individuals of different ages. Such mixed populations are not suitable for selection for growth. The non-synchronous spawning in tilapia makes mass selection more difficult since the larger individuals are likely to be progenies of earlier spawns, rather than faster growers (Hulata et al., 1986). Fish breeders might also inadvertently select for cannibalistic fish as those fish display desirable traits such as fast growth rate and robustness (Li and Mathias, 1982). Variation in size due to non-heritable effects such as asynchronous spawning and size dependent cannibalism will, therefore, increase the phenotypic variance, mask the genetic components of growth and can often lead to marginal or no response (Doyle and Talbot, 1986; Ruzzante, 1993; Lutz, 2004).

Small-scale farmers and hatcheries in developing countries are even more constrained by access to knowledge of genetic management and commit many mistakes which have been often blamed for the failure of mass selection schemes. A typical practice at harvest among small-scale farmers in Africa is to sell or eat all fish of certain size, leaving smaller stunted individuals to be either sold as fingerlings to other farmers or continue growing them in their own ponds. This inadvertent selection of smaller adults is tantamount to selection for slow growth and early sexual maturation (Brummett et al., 2004). Considering the heritability for body weight (0.38-60) and female GSI (Gonado-Somatic Index) (0.25) reported in Charo et al. (2006), such an unintentional negative selection will not only result in no genetic progress but may also lead to deterioration of stocks in the long run. Anecdotal and empirical evidence exists for substantial (up to 40%) declines in growth among *Oreochromis* populations domesticated in both large and small-scale fish farms in Africa (Brummett and Ponzoni, 2004).

Overall, simple and unstructured mass selection schemes based on mass-spawning are often practiced but have often resulted in no or marginal genetic progress. In order to refine the design and improve the efficiency of mass selection based on natural mating, it is crucial to have a better understanding on: reproduction under mass spawning conditions; the actual contribution of broodstock to the next generation; measures to constrain inbreeding and increase effective population size, synchronization of spawning and management of size heterogeneity to minimize size dependent cannibalism (See Figure 1).

3. Natural reproductive behaviour of *O. niloticus*

The mating system of the African cichlid fish *Oreochromis spp.* resembles that of other lekking animals; males defend mating territories where the spawning pits they dig are sites for mating and oviposition (Baerends and Baerends-van Roon, 1950; Fryer and Iles, 1972). The female lays her eggs in the spawning pit after which they are fertilized by the male. Under natural conditions, females may encounter solitary or aggregated males and experience varying degrees of male interference and competition during courtship and spawning (Nelson, 1995). After fertilization of the eggs, the female picks up the eggs in her buccal cavity. Thereafter, the female leaves the spawning pit and rears her clutch until the fry are free-swimming. The male continues to defend the pit and attract other females for mating (Fryer and Iles, 1972; Rana, 1988).

4. Commercial fry production in *O. niloticus*

In intensive hatcheries, fry production of *O. niloticus* is typically achieved by stocking of males and females in tanks or in hapas suspended in fertilized ponds, harvesting seed from the mouths of females and incubating them artificially in tank-based hatcheries (Little et al., 1995). Sexually mature tilapias generally undergo successive reproductive cycles at intervals of 3–6 weeks. Theoretically, this could lead to almost continuous production of fry, assuming that seasonal environmental variation is minimal. However, the reproduction in *O. niloticus* is characterized by an asynchronous ovarian cycle (Rana, 1990). This has two implications. First, asynchronous spawning of females will result in competition by males for access to these spawning females (Grant et al., 1995). Secondly, it leads to episodic fry production (Jalabert and Zohar, 1982) which in turn might cause size-dependent competition and cannibalism (Figure 1).

4.1. Reproductive competition

When females spawn asynchronously, individual males with high competitive ability can potentially acquire access to a disproportionately large share of the total number of females that spawn in a given period of time (Grant et al., 1995). Mating systems can strongly influence effective population size (N_e), by affecting the likelihood that an individual will reproduce (Nunney, 1991), by influencing the variance in individual reproductive success within one or both sexes (Nunney, 1993) and by affecting the number of mates per individual (Sugg and Chesser, 1994). Higher fecundity and higher reproductive success of a few parents may result in a quite high variance in progeny number and consequently small within-generation N_e and low N_e/N ratio (Waples, 1998). When N_e is low, there is considerable risk that the amount of genetic variation in the population will be reduced by genetic drift (Hedrick, 2005). There is a direct relationship between N_e and inbreeding, such that N_e is equal to $1/(2\Delta F)$, where ΔF is the per-generation rate of inbreeding (Falconer and Mackay, 1996). To ensure the long-term success of breeding programmes, the maintenance of genetic variation is very important (Hedrick et al., 1986). Reduced genetic variability as a result of genetic drift limits the opportunity for future genetic improvement.

4.2. Size heterogeneity and cannibalism

Asynchronous reproductive cycles in *O. niloticus* also lead to episodic fry production. In practice, hatcheries combine fry of different families hatched at different times. This will result in groups of fry showing a wide range in size (Jalabert and Zohar, 1982; Coward and Bromage, 2000). Stocking of heterogeneous fry groups leads to social dominance which often results in aggressive and cannibalistic behaviour (Hecht and Appelbaum, 1988). In addition to intercohort competition, intracohort cannibalism was found in groups in which large size differences become established within age cohorts of *O. niloticus* (Pantastico et al., 1988). Size-dependent cannibalism in *Oreochromis spp*. may account for an average loss of 10-35% of fry stocked during the first 50 days of rearing (Macintosh and De Silva, 1984). Initial size advantages resulting from environmental effects (including cannibalism) can become exaggerated over time and obscure differences attributable to genetic superiority or inferiority (Lutz, 2004). Therefore, size-dependent cannibalism not only causes direct economic losses but can also have long term negative effects on the genetic gain of genetic improvement programmes (See Figure 1).

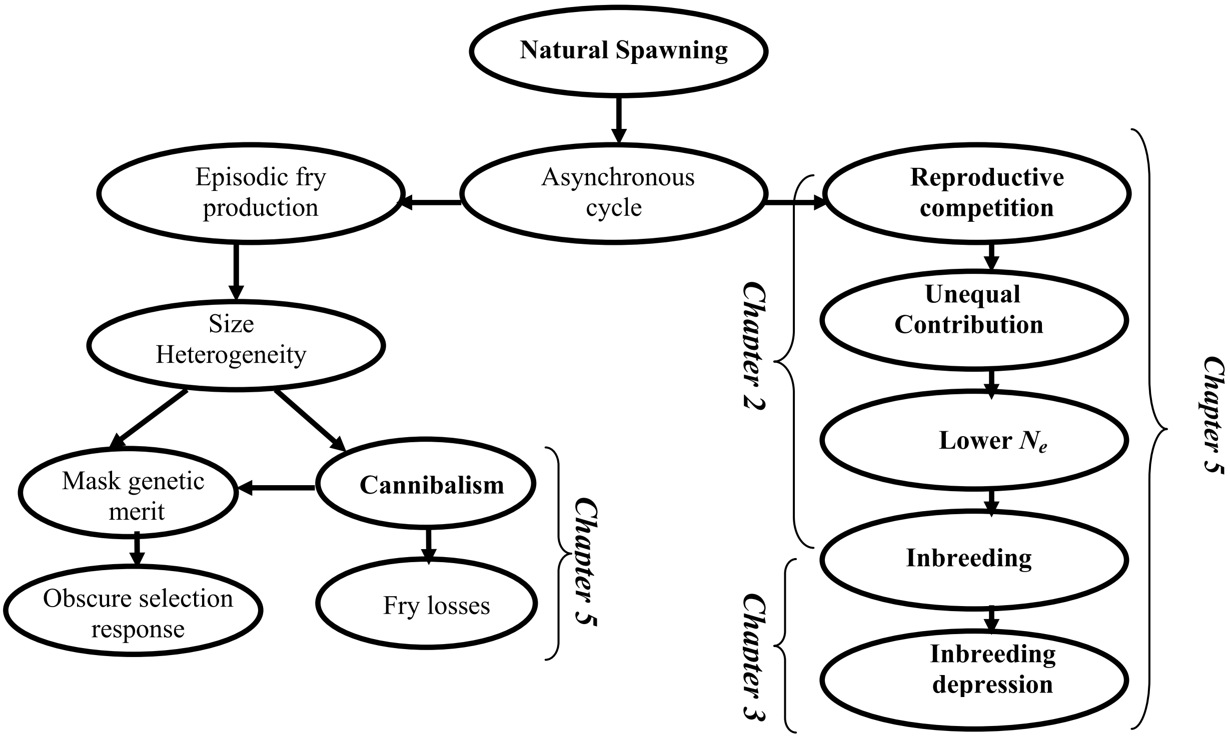

Figure 1. Schematic representation of the research frame work

5. Aim and outline this thesis

The aim of this thesis was to gain thorough knowledge and understanding of the natural mating systems in *O. niloticus* and its consequences for genetic improvement programmes. The outline of this thesis and the relationship between the different chapters are presented in Figure 1. In **Chapter 2**, microsatellite markers are used to determine parentage and to investigate the mating system of mass-spawning *O. niloticus* under semi-natural conditions in breeding hapas. Two groups of 37 tilapias were stocked at female to male sex ratio of 2. Reproductive success of both males and females was determined and the per-generation rate of inbreeding and effective population size was estimated. As we found substantial estimates of the rate of inbreeding, in **Chapter 3** we investigated the effects of different levels of inbreeding on survival and on body weight at both early and later life stages. In addition the effect of inbreeding on fluctuating asymmetry at later stage in life was also investigated. In **Chapter 4** microsatellite markers were used to investigate the effects of different levels of inbreeding on the individual reproductive success in *O. niloticus.* Fish were stocked at a female to male sex ratio of 1 and 3 to compare reproductive success of males under different competitive conditions. The estimates of inbreeding depression in reproductive success for both males and females are given.

In **Chapter 5,** factors influencing size-dependent cannibalism in Nile tilapia, *Oreochromis niloticus* are investigated in controlled conditions. The aim of this chapter is to derive simple rules for management of heterogeneous fry groups by which cannibalism can be minimized

Finally in **Chapter 6**, the results of this thesis are discussed in view of their implications to aquaculture breeding schemes and natural populations.

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Chapter 2

Mating systems and male reproductive success in Nile tilapia (*Oreochromis niloticus*) in breeding hapas: a microsatellite analysis

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***Aquaculture* 256 (2006), 148-158**

Abstract

Fry production in Nile tilapia, *Oreochromis niloticus* is typically achieved by mass-spawning of males and females stocked in large hapas suspended in ponds. In such spawning aggregations, territorial behaviour and reproductive competition among males may lead to a large variance in reproductive success among individual males. We analyzed parentage and quantified male reproductive success of *O. niloticus* under commercial hatchery conditions in two breeding hapas each stocked with 12 males and 25 females. Eleven microsatellite markers were used to estimate the reproductive success of individual males and females. In total, 760 offspring from 76 spawnings were included in the paternity analysis. Mating patterns in Nile tilapia ranged from single pair to promiscuous mating. Multiple paternity was detected in 70% of the broods, with up to 4 males fertilizing a single clutch. Multiple maternity was also detected in over 30% of the clutches analyzed. There was a very high variance in male reproductive success, with one third of males siring more than 70% of the offspring. Male condition factor had a significant effect on reproductive success with larger males siring a large proportion of offspring. The high reproductive variance resulted in a N_e of around 16 for hapas A and B, and a low N_e/N ratio, 0.43 and 0.45 for hapas A and B respectively. The rate of inbreeding, ΔF, for each hapa, was estimated to be around 3.0% per generation, which is about twice the inbreeding expected in an idealized population of the same census size. When designing fish breeding programmes based on mass-spawning, the higher inbreeding and lower effective population size should be taken into consideration.

1. Introduction

Male reproductive behaviour may be extremely diverse, both within species and between species. Among vertebrate classes, fish exhibit by far the greatest variability in competitive and cooperative behaviours in male reproduction (Taborsky, 2001). Reproductive competition may lead to a large variance in reproductive success among individual males thereby contributing to a low effective population size (Bekkevold et al., 2002). The genetic consequence of unbalanced contributions of parents is of concern for selective breeding schemes which use mass spawning (Boudry et al., 2002). In those schemes inbreeding is likely to occur, leading to decreased in performance (Kincaid, 1976). Furthermore, reduced genetic variability limits the possibility of future genetic improvement by selective breeding.

Tilapias are among the most important freshwater species with the greatest production expansion in aquaculture in recent years (Fitzsimmons and Gonzalez, 2005). In intensive hatcheries, fry production in *O. niloticus* is typically achieved by stocking of males and females in large hapas suspended in fertilized ponds, harvesting seed from the mouths of females and incubating them artificially in tank-based hatcheries (Little et al., 1995). *O. niloticus* is a lek-spawning fish: each male builds and defends a territory within a defined spawning area (a 'lek') where oviposition of eggs by females takes place (Rana, 1988). A characteristic feature of leks is high variance in reproductive success among males within an aggregation, with more attractive and dominant males gaining a disproportionate share of matings (Hoglund and Alatalo, 1995: quoted in Bekkevold et al., 2002). Considerable effort has been devoted to understand why success is distributed so unevenly among males, including the roles of male-male competition and female choice (Beehler and Foster, 1988; Reynolds and Gross, 1990; Stillman et al., 1993).

Whereas behavioural observations indicate that the mating system of the African cichlid fish *O. mossambicus* resembles that of other lekking animals (Nelson, 1995), no genetic study of the mating system of *Oreochromis* species has been carried out. Furthermore, no direct quantification of reproductive success and parental contribution to the next generation has been previously investigated in Nile tilapia. In this study, we used eleven highly polymorphic microsatellite markers to analyze parentage of offspring from several spawnings to genetically characterize the mating systems in *O. niloticus* and to quantify individual males' reproductive success in breeding hapas. We determine the variance in individual reproductive success and discuss the possible factors contributing to this large variance. Finally, the consequence of mating systems and parental contribution on effective population size is discussed in relation to breeding programmes which employ mass spawning for fry production.

2. Materials and Methods

Fish

The spawning experiments were carried out at the experimental station of The WorldFish Center, Abbassa, Egypt. Grandparents of the experimental fish were produced in the spring of 2000 from all possible diallele crosses between four Egyptian strains of *O. niloticus* (Rezk et al., 2002). Parental fish, which consisted of 24 males and 50 females (Females: average = 73.09g, CV =17.5%; males: average = 73.78 g, CV = 19%), were randomly selected from this population maintained at the research station. Females and males were separately conditioned for two weeks prior to stocking in the breeding hapas by feeding them twice daily at a feeding rate of 3% body weight per day with 3mm floating pellets (40% crude protein, Alexandria Oil and Soap Company, Kafr El-Sheikh, Egypt).

Experimental set up

The spawning experiments were carried out in the breeding season of 2003. Prior to the experiment, all fish were anesthetized with tricaine methanesulfonate (MS-222) and tagged with Floy® tags between the dorsal fin and lateral line. All parental fish were measured for total body weight (W, nearest 0.1 g); total length (TL), standard length (SL), head length (HL), head width (HW) and body depth (BD). TL and SL were measured as the distance from the tip of the snout to the end of the caudal fin and hypural bone respectively; HL was measured from the tip of the snout to the end of the opercle; HW is the maximum head width near the opercle and BD is the maximum body depth measured dorso-ventrally just anterior to the dorsal fin. About 0.5 ml of blood was collected from all parental fish by caudal puncture using a hypodermic needle rinsed with Na_2EDTA. Two 8 x 2 x 1 m^3 (length x width x depth) hapa enclosures were installed in two concrete- walled ponds with mud bottom (dimension: 50 x 5 x 1m^3; the floor of the hapas was sitting on the pond bottom). Broodstock were divided into two random groups of 25 females and 12 males and were stocked in either one of two hapas. Two days after stocking, one male died in hapa A and was replaced by another one. Broodstock were allowed to spawn naturally for a period of ten days after which checking for eggs in the mouth of breeders commenced. Collection of eggs was carried out on a weekly basis for a duration of three weeks after which males and females were separated for a reconditioning period of two weeks by feeding them with high protein diet (40% crude protein). After the reconditioning period, breeders were re-stocked into their original hapas and were allowed to spawn again for a period of about ten days after which 3 more weekly collections of eggs were made.

Egg collection and incubation

All females were checked during each egg collection. Females carrying eggs in the buccal cavity were identified with the tag number and eggs were collected from the mouth of each incubating female into a plastic bowl. The unhatched eggs were then rinsed and counted before transferring them to the artificial incubators.

Fertilized eggs were incubated in 20-l aquaria with continuous aeration to keep the eggs in constant motion and to ensure sufficient oxygen supply needed for hatching of eggs. To control fungal infection, the water was treated with 30 ppm formalin for the first day and with 20 ppm formalin for the subsequent days. Formalin treatments were stopped when the embryos reached the eyed stage. In addition, eggs were inspected daily and unfertilized or dead eggs were removed as the presence of spoiled eggs may cause infection and loss of the whole spawn. Fry were reared in the incubation system till the yolk sac stage. Hatching percentages and fry survival was recorded for all families. Finally, ten yolk sac fry per spawn were randomly sampled for DNA extraction and genetic analysis.

DNA extraction and microsatellite analysis

Genomic DNA was extracted from blood of parental fish and whole yolk sac larvae. DNA was isolated from blood of parental fish using the PUREGENE kit (Gentra Systems, Minneapolis, MN, USA) following the manufacturers instructions for non-mammalian blood. Yolk sac fry were overdosed with MS-222 (tricaine methanesulfonate). The yolk sac was then removed using a scalpel and embryos were placed in sterile 1.5 ml microcentrifuge tubes. Extraction of DNA from whole larvae was also carried out using the same kit and protocol as for non-mammalian blood. The amount of re-hydrated DNA solution was adjusted to yield final DNA concentrations of 5–10 μg/ml. Eighteen microsatellite markers from the database at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) were screened. Based on their level of heterozygosity and genotyping reliability, the best eleven microsatellite markers (*UNH160, UNH169, UNH190, UNH203, UNH212, UNH222, UNH123, UNH178, UNH208, UNH214, UNH231*) were selected for genotyping all parental fish and offspring. PCR cycling conditions were as follows: 5 min at 95 °C, 35 cycles of 30 s at annealing temperature (45–60 °C), and 30s at 72 °C, followed by a final elongation step of 4 min at 72 °C. Amplified products were combined in multiplex groups of five to six markers, and electrophoresed on 6% polyacrylamide gel on the ABI prism 373 DNA sequencer (Applied Biosystems, CA, USA). Fragment sizes were calculated relative to the TAMRA 350 size standard. Genotype results were analyzed using GENESCAN version 3.1 and GENOTYPER version 2.5 (Perkin-Elmer, Boston, MA, USA).

Statistical analysis

Parentage analysis: Allocation of offspring to their putative parental pairs was performed using PAPA vers. 2.0. (Package for analysis of parental allocation). PAPA is a computer programme that performs parental allocation by calculating the likelihood of each potential parental pair. Given an offspring genotype, the likelihood of parental pair of genotypes is defined as the probability of this pair breeding the offspring genotype among all its possible descents (Duchesne et al., 2002). The probability of erroneous scoring of allelic size (such as allele designation, stutter-related scoring, or upper allele dropout errors) in an individual genotype must be considered when conducting maximum-likelihood parentage analysis (Sancristobal and Chevalet, 1997). In our parentage analysis we assumed an error rate of 0.02 and a uniform distribution of the errors. To test the parental assignment and to estimate the efficiency of the loci used in parental allocation, a random sample of 10,000 simulated offspring were generated from all parental male and female genotypes. These simulations were conducted by adding loci from the most polymorphic to the least polymorphic one.

Condition factor of males: Condition factor (K) was calculated as $K = W\ (g)/SL^{2.93}$, whereby the exponent in the formula is the slope of the regression of $Log_{10}W$ on $Log_{10}SL$ (Bolger and Connolly, 1989).

Male reproductive success: Male reproductive success was calculated for each male as the proportion of offspring sired by a male in a single spawning. All correctly allocated offspring were included in this analysis. Genetically effective paternity frequency was calculated for each spawning following Bekkevold et al. (2002). The same formula was also used to compute the genetically effective maternity frequency.

$$PF = 1 \Big/ \sum_{i=1}^{n} p_i^{\,2}$$

Where PF is genetically effective paternity frequency

p_i is the proportion of offspring sired by the i^{th} male in a given clutch

n is number of males involved in fertilizing a clutch

For each spawning, the reproductive success of each male in a hapa was considered to be in the range of 0 to 1; whereby 0 is the case in which a male had sired none of the offspring and 1 in which a male had sired all the offspring in that spawning. The effects of hapa, condition factor of males, date of sampling on the reproductive success of males was then investigated using generalized, linear model procedure (GENMOD) of SAS (1989). A generalized linear model with binomial errors and logit link function was used as male reproductive success was calculated as the proportion of offspring sired by a male within a single spawning. A basic model was fitted with an effect of hapa (I = 1, 2), and sampling date (j = 1 till 6). In order to test the relative importance of the explanatory variables, the basic model was run by adding one additional variable at a time and in each successive run another variable was replaced (condition factor, weight and morphometric measurements such as TL, BD and HL).

Calculation of effective population size (N_e) and rate of inbreeding (ΔF): The effective population size for each hapa was calculated after Wright (1938) as:

$$N_e = 4N_{em}N_{ef} \big/ (N_{em} + N_{ef})$$

Where N_{em} and N_{ef} are the effective number of males and females, respectively. N_{em} and N_{ef} were calculated following Lande and Barrowclough (1987):

$$N_{em} = (N_m \bar{k}_m - 1) \Big/ [\bar{k}_m + (\sigma_{km}^{\;2} / \bar{k}_m) - 1]$$

Where N_m is the actual number of males; $\bar{k}_m$ is the average number of offspring sired by an individual male and $\sigma_{km}^{\;2}$ is the variance of k_m. The same formula was used to compute the effective number of females.

The rate of inbreeding, ΔF was calculated after Brown et al. (2005) using the following formula

$$\Delta F = \frac{1}{2}\sum_{Parents} c_i^{\,2} - \frac{1}{4}(\bar{c}_m)^2 - \frac{1}{4}(\bar{c}_f)^2$$

Where c_i is the fractional contribution of each parent, $\bar{c}_m$ is the average fractional contribution of males, 1/ ($2C_m$), and $\bar{c}_f$ is the average fractional contribution of females, 1/ ($2C_f$)

3. Results

Female spawning

The total number of spawns collected over the experimental period was 47 and 29 for hapa A and hapa B respectively. About 92% of all females stocked in both hapas spawned and about 44% had more than one spawning. The average (± SD) female fecundity and hatching percentage over all the spawnings and both hapas combined was 523 (± 184) and 74 (± 16) respectively.

Parentage analysis

Parental allocation was highly reliable and efficient as demonstrated by the results of the reassignment simulations. About 97.0% of the simulated offspring could be assigned to their correct parental pairs based on information from only five loci. With all the eleven loci, almost 100% of correct allocation could be attained (Figure 1). Parental assignment was then performed on a total of 760 real offspring collected from 76 spawnings (Hapa A = 47; Hapa B = 29). Over all, 97.7% and 99.7% of all offspring were unambiguously allocated to their putative parental pairs based on information from five and eleven loci respectively. In hapa A, 467 out of the 470 (99.4%) offspring were unambiguously allocated to their putative parents. Three offspring could not be allocated to a single pair of parents because the samples of these offspring amplified for only three of the eleven markers. These three offspring were not considered in the subsequent analysis. In hapa B, all 290 offspring were unambiguously allocated to their putative parental pairs. To confirm if the custodian mothers are the real mothers of the offspring, the mothers allocated to offspring using PAPA 2.0 (genetically deduced) were cross-checked with the tag numbers of custodian mothers which were mouthbrooding the eggs at the time of collection. All the custodian mothers except two were confirmed to be the real mothers of the offspring they were incubating at the time of collection. In these two clutches, genotypes of three and four mothers were found and none of the juveniles were dammed by the custodian mothers who held them; these two clutches were fertilized by one and five males respectively. In the subsequent analysis, the genetic mothers and not the custodian mothers were taken as the mothers of the offspring in those clutches.

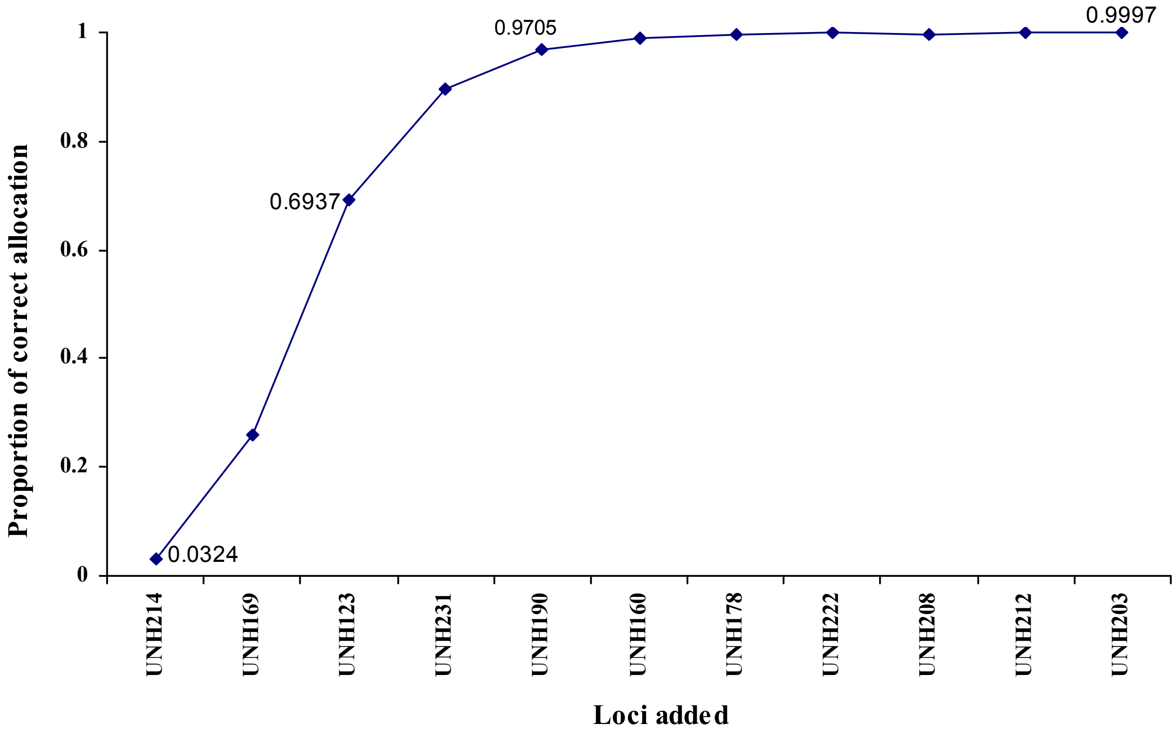

Figure 1. Reassignment procedure of 10,000 simulated offspring to their parental pairs adding loci from the most to the least polymorphic one

Table 1 depicts the occurrence of different mating systems in Nile tilapia. Only 21.05% of the spawnings were a result of mating between a single male and a single female (hereafter: single pair spawning). Multiple males (hereafter: multiple paternity) were involved in fertilizing a single clutch in 35 of the 76 spawnings (46.05%). In both hapas A and B, genotypes of up to 4 males were detected in a single clutch of eggs. Six out of 76 spawning (7.89%) were found in which the custodian female was the dam of most but not all of the eggs she tended. The proportion of juveniles not dammed by the custodian mother ranged from 10 -30%. These clutches had embryos from multiple mothers fertilized by a single male (hereafter: multiple maternity); 19 out of 76 clutches (25%) had embryos from multiple mothers which were fertilized by multiple males (hereafter: promiscuity).

The relative contribution of males in siring offspring in both hapas is shown in Figure 2 and 3. In hapa A, the male that died two days after stocking and its offspring (three in number) were also excluded from subsequent analysis. In both hapas A and B, there was clear evidence of male dominance. Of the twelve males, four sired about 72% and 75% of the offspring in hapas A and B respectively. The average contribution of each of the six least dominant males was 1% and 1.3% in hapas A and B respectively. The average proportion of offspring sired by different males ranking from the most to the least successful, and the genetically effective paternity frequencies for the spawnings in both hapa A and B are shown in Table 2. The average number of fathers contributing to a spawning for hapas A and B were 1.89 (SE = 0.16) and 2.09 (SE = 0.20) respectively. The mean genetically effective paternity frequency (± SE) for hapas A and B respectively was 1.54 (0.10) and 1.51 (0.13); the genetically effective paternity frequency for hapas A and B combined was 1.52 (0.08).

Compared to the unbalanced contribution in males, about 92% of all females stocked in both hapas spawned. The average proportion of offspring contributed by different females in a single clutch ranking from the female contributing most of the offspring to the least one, and the genetically effective maternity frequencies for all the spawnings in both hapa A and B are shown in Table 3. For hapa A and B together, about 89% of the spawnings constitute of embryos contributed by a single female. The remaining 11% included embryos from more than one female. For both hapas combined, average number of mothers contributing to a single clutch was about 1.16 (SE = 0.07) and the genetically effective frequency of mothers was 1.07 (SE = 0.03).

Table 1. Different mating systems in *O. niloticus*.

Hapa	Single-pair mating	Multiple paternity	Multiple maternity	promiscuity	Total
A	10 (21.28%)	20 (42.55%)	5 (10.64%)	12 (25.53%)	47
B	6 (20.69%)	15 (51.72%)	1 (3.45%)	7 (24.14%)	29
Total	16 (21.05%)	35 (46.05%)	6(6.58%)	19 (25.00%)	76

Numbers in brackets are percentages of the total spawns

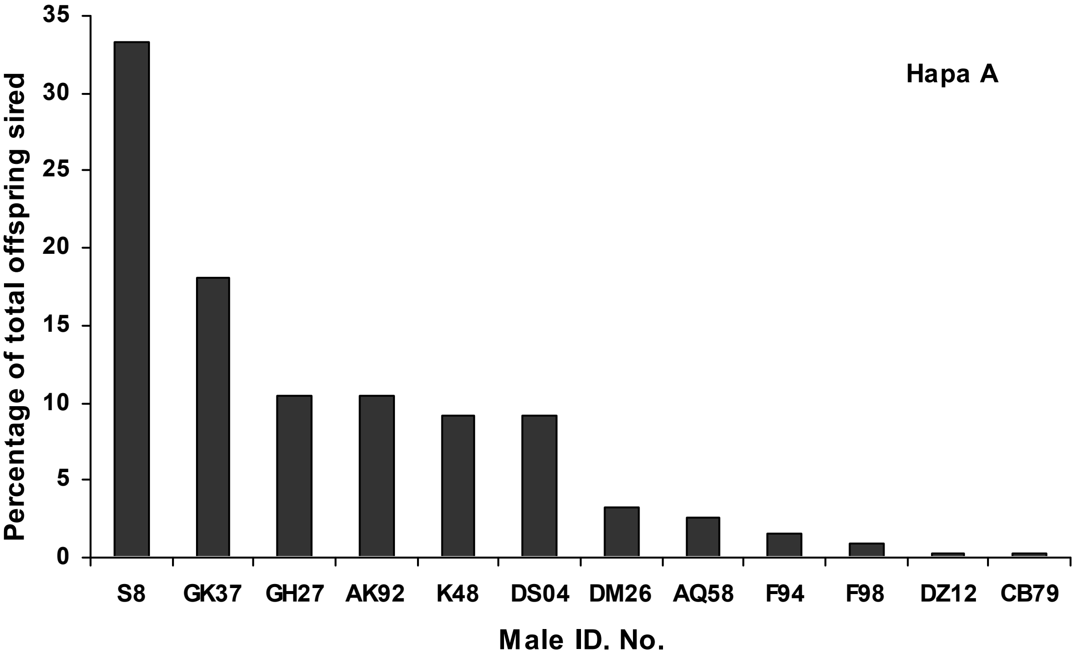

Figure 2. Percentage of offspring sired by males in hapa A

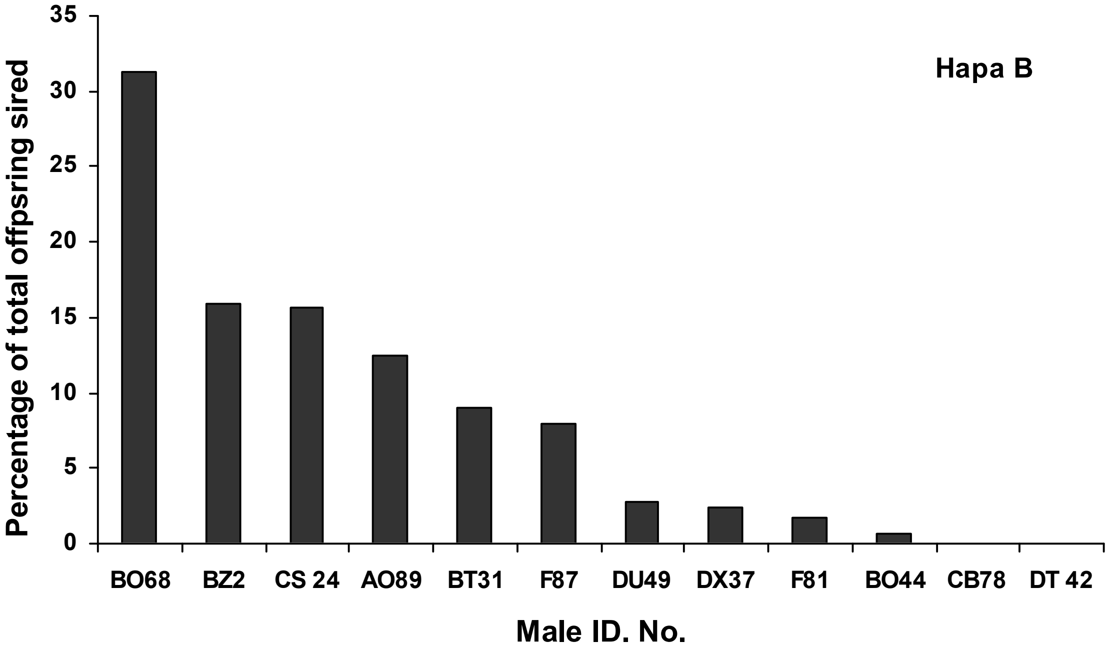

Figure 3. Percentage of offspring sired by males in hapa B

Male reproductive success

The GENMOD analysis of factors affecting individual male reproductive success showed that male condition factor ($P < 0.0001$), body weight ($P < 0.0001$), total length ($P < 0.0001$), body depth ($P < 0.0001$) and head length ($P < 0.0001$) within hapa had a significant effects on reproductive success of males. The hapa enclosures ($P < 0.0180$) differed with respect to patterns of male reproductive success but sampling dates did not have significant effect on the reproductive success of individual males (Table 4).

Table 2. Means ± SD paternity proportions for spawnings in which from one up to four males were detected to contribute sperm.

No. of males participating in spawning	No. of spawnings (% of total)	Paternity proportion of most successful male	Paternity proportion of second-most successful male	Paternity proportion of third-most successful male	Paternity proportion of the least successful male	Genetically effective paternity
Hapa A						
1	15 (42.86%)	1	-	-	-	1.00 ± 0.00
2	12 (34.29%)	0.74 ± 0.12	0.26 ± 0.12	-	-	1.61 ± 0.28
3	5 (14.29%)	0.58 ± 0.11	0.26 ± 0.06	0.16 ± 0.09	-	2.28 ± 0.40
4	3 (8.57%)	0.53 ± 0.06	0.23 ± 0.06	0.13 ± 0.06	0.10 ± 0.00	3.73 ± 0.04
Hapa B						
1	7 (31.82%)	1	-	-	-	1 ± 0.00
2	7 (31.82%)	0.81 ± 0.12	0.19 ± 0.12	-	-	1.43 ± 0.29
3	7 (31.82%)	0.70 ± 0.13	0.20 ± 0.13	0.10 ± 0.00	-	1.83 ± 0.40
4	1 (4.55%)	0.4	0.3	0.2	0.1	3.33

The distribution of paternity is given for each male ranked from most successful to least successful male respectively. The average genetically effective number of fathers is also given. Promiscuous matings were not included for calculation of effective paternity frequency (n =12 and 7 for hapa A and B respectively)

Effective population size

Contributions of parents over the different sampling periods were pooled to estimate the effective population size in hapa A and B. The effective numbers males and females for both hapas are shown in Table 5. Less than 50% of the males and about 70% of females were contributing to the next generation in both hapa A and B. Similarly, the variance of male contribution (σ_{km}^2) was greater than the variance of female contribution (σ_{kf}^2) in both hapas indicating that male contribution was more skewed than that of females. N_e for was similar for both hapas with a value of around 16. The N_e/N ratio of 0.43 and 0.45 for hapa A and B respectively indicate that less than 50% of parents were contributing to the next generation. The rate of inbreeding within each hapa, ΔF, was estimated at around 3.0% per generation.

Table 3. The average ± SD maternity proportions in which a single clutch of eggs were dammed by up to three females.

No. spawning females	No. of spawnings (% of total)	Maternity proportion of most successful female	Maternity proportion of second-most successful female	Maternity proportion of third-most successful female	Genetically effective no. of mothers to a spawning
Hapa A					
1	30 (85.71)	1	-	-	1.00 ± 0.0
2	2 (5.71%)	0.80 ± 0.14	0.20 ± 0.14	-	1.47 ± 0.36
3	3 (8.57%)	0.73 ± 0.06	0.17 ± 0.06	0.1 ± 0.00	1.74 ± 0.19
Hapa B					
1	21 (95.45%)	1	-	-	1
2	1 (4.55%)	0.90	0.10	-	1.22

Contributing females are ranked from the one contributing most of the eggs to the one contributing the least. Genetically effective number of mothers is also given. Promiscuous matings were not considered for calculation of effective maternity frequency (n =12 and 7 for hapa A and B respectively)

4. Discussion

Parentage analysis

The parental allocation procedure of both simulated and real offspring was very efficient in establishing the offspring-parent relationship. About 97-98% of all offspring were unambiguously assigned to their correct parental pairs based on only the five most informative loci. The allocation results of both the simulated and real offspring are in agreement to each other and with result of the a priori assignment expectations based on simulation assignment and parentage model of Bernatchez and Duchesne (2000). Predictive models allow the adjustment a priori of the required level resolution and consequently assist optimization of the cost-benefit ratio in the use of molecular markers. Predictions of power of microsatellite markers for parental assignment assume absence of genotyping errors, mutation or non-amplifying or ‘null’ alleles (Villanueva et al., 2002). Violation of such assumptions can lead to a lower allocation efficiency and frequent mismatches of offspring to their true parents. Our allocation results show that a satisfactory level of parental assignment could be attained with only five loci, however, the use of 6-8 microsatellites would ensure higher allocation efficiency and would overcome the assignment failures arising from genotyping errors and null alleles.

Table 4. General linear model analysis (type III) on the effect of hapa, sampling date, male condition factor, male weight, TL, BD and HL on male reproductive success.

Effects	*df*	χ^2	*P* -value
Hapa	1	5.60	< 0.0180
Sampling date	5	0.28	0.9980
Condition factor*	1	403.36	< 0.0001
Weight*	1	296.82	< 0.0001
Total length*	1	200.41	< 0.0001
Body depth*	1	386.11	< 0.0001
Head length*	1	261.56	< 0.0001

The basic model (hapa, sampling period) was run by adding another explanatory variable (indicated by *) at a time and replacing it with another in the subsequent analysis.

Mating systems

Fish have some of the most complex mating systems known in the animal kingdom (Neff, 2001). Our results show a diversity of reproductive pattern ranging from single pair to promiscuous matings. Multiple paternity in mouth brooding tilapias (at that time termed as Sarotherodon) has been documented as early as 1981 using allozymes (Hulata et al., 1981). Recently, microsatellite-based paternity analyses in several cichlid species have documented multiple paternity of broods, with up to six males fertilizing a single clutch (Parker and Kornfield., 1996; Kellogg et al., 1995). Similarly, the parentage analysis in our study has shown that up to four males fertilized a single clutch of eggs. While males are expected to be promiscuous because male reproductive success is directly related to the number of females inseminated, the adaptive significance of females copulating with multiple males is less clear

(Parker, 1992). Female multiple mating may be male driven, but females often directly seek for copulations from a number of males, and it is becoming increasingly clear that many females in a wide range of taxa are genetically polyandrous (Gowaty, 1994). Females also gain any of several fitness advantages by mating with multiple males. Such benefits include fertilization insurance against male sterility, access to more or better quality territories, success in 'prospecting' better genes for her progeny, production of broods with more diverse and potentially adaptive genotypic arrays, and avoidance of inbreeding depression if some of her matings might be with close kin (Avise et al., 2002). However, multiple mating of females could also simply be a matter of re-spawning. When a female fails to spawn all of her eggs during a single mating bout, she may spawn again in a different nest and with a different male leading to the siring of her eggs by multiple males (DeWoody and Avise, 2001). Our study documented five clutches in which the custodian mother dammed most but not all of the offspring she tended. In those clutches, the proportion of offspring not dammed by the custodian mother was between 10 to 30%. In addition, two custodian females carrying the eggs dammed none of their apparent offspring. These maternal exclusions were based on eleven highly polymorphic loci and the possibility of wrong parental assignment could be ruled out. Intraspecific brood mixing, shuffling of conspecific broods, is a cryptic phenomenon in mouth-brooders documented by microsatellite markers. In four of the six orally brooded cohorts of fry examined in a Lake Malawi cichlid (*Protomelas spilopterus*), the proportions of juveniles not dammed by the female who held them ranged from 6% to 65% (Kellogg et al., 1998). The origin of brood mixing has traditionally been explained in several ways: (1) parents carry the offspring in their mouths and put some under the care of foster parents termed as 'farming-out' (McKaye et al., 1992); (2) foster parents kidnap the young (McKaye and McKaye, 1977); (3) aggressive behaviour between parents of two adjacent broods results in the winner's acquisition of the loser's brood (Lewis, 1980) and (4) young of guarded broods close to each other simply mix (McKaye, 1977); and (5) deserted or stray young intrude into unrelated broods (Ribbink et al., 1980). DeWoody and Avise (2001) suggested that genotypic incompatibility between an embryo and its guardian may also result from 'egg thievery'. Several of the possible explanations for the origin and significance of brood mixing remain highly speculative and the true origin of brood mixing remains unclear because of the difficulty of observing the process of mixing (Kellogg et al., 1998). High fish density, space limitation and inability of fish to construct nests in artificial hapa environment might have led to egg mixing and shuffling of broods.

Table 5. Number of broodstock (*N*), averaged offspring count of male (k_m) and female (k_f) parents, variance of male and female contributions (σ_{km}^2, σ_{kf}^2 respectively), effective numbers of males and females (N_{em}, N_{ef} respectively), effective population size (N_e), coefficient of inbreeding (ΔF)and the ratio of N_e/N for hapa A and B.

Hapa	N	k_m	k_f	σ_{km}^2	σ_{kf}^2	N_{em}	N_{ef}	N_e	ΔF	N_e/N
A	37	38.3	19.33	2094.06	148.84	4.99	17.78	15.60	0.0309	0.43
B	37	24	12	724.72	85.83	5.39	15.8	16.08	0.0301	0.45

Male reproductive success

Parentage analysis in this experiment revealed that there is a large variance in the reproductive success of males and that one third of males sired more than 70% offspring in both hapas. This is a striking feature of many animal species in which few males obtain most of the matings, while the rest have little or no success. Male reproductive competition for fertilization has been documented in a rapidly increasing number of genetic paternity studies of a wide range of fish species (DeWoody and Avise, 2001). Variation in male success is most pronounced in leks, in which 10-20% of males often obtain 70-80% of the matings (Wiley, 1991). Reproductive success in lekking species is dependent on male-male interaction and female mate choice (Johnstone and Earn, 1999). Some authors suggest that differences between male's mating success at a lek are largely the result of female preferences (Andersson, 1992); others suggest that male –male competition is more important in determining male mating success (LeCroy, 1981; Beehler and Foster, 1988). Male-male competition could not be observed directly in our study. Even so, previous observations of tilapia reproductive behaviour (Nelson, 1995) together with our study showing a correlation between male condition factor/weight and reproductive success, leads to the hypothesis that males experience reproductive competition. Male condition factor and weight had a significant effect on reproductive success with males of higher condition factor/weight siring a large proportion of offspring. Rakitin et al. (1999) reported that the most successful cod male had a greater condition factor (*K*) and also higher spermatocrit than the least successful male, suggesting that sire condition factor is potentially a critical factor determining sperm fertilization potency. It is, however, difficult to disentangle the relative effects of female mating preference from male –male competition on the reproductive success of males. Several studies have evaluated female mate choice among Old and New World cichlids and identified male characters such as bower size, egg-spot numbers and male body size that lead to non-random mate selections (Noonan, 1983; McKaye et al., 1990). Female *O. mossambicus* made choices based on two criteria under different circumstances: a spawning site characteristic, spawning pit size and a morphological feature of males, body length (Nelson, 1995). The assumption that bigger males have advantages in male-male competition or in female mate choice has been challenged. Bekkevold et al. (2002) argues that larger males might have been able to sire more offspring simply by having larger ejaculates, which swamps those of smaller males, rather than because they acquire more matings with females. The relative importance of male-male competition and female choice to the skewed mating success in tilapia is not clearly understood and merits further investigation.

Effective population size

Effective populations size (N_e) depends strongly on mating systems. The mating system is a primary determinant of variation in male mating success. Under extreme polygyny (with very few males obtaining almost all of the matings), high variance in reproductive success results in a low effective population size. When obvious inequalities in reproductive success occur, N_e is always much smaller than in comparable monogamous populations (Nunney, 1993). Low N_e/N ratios could be the result of high variance in reproductive success, which decreases the genetically effective size of a population without affecting census population. This factor is especially important in highly fecund species in which most of the mortality occurs during the egg and larval stages (Hedgecock, 1994). Boudry et al. (2002) also demonstrated that the effective population size is strongly reduced in oysters, because of unbalanced parental contribution, even when gametic contributions are balanced. Our results are in agreement with these findings. Large variances in male contribution (σ_{km}^2) together with the small number of contributing males were the factors that led to the reduced magnitude of N_e. In addition, the skewed reproductive success and lower N_e resulted in an inbreeding rate of around 3% per generation which is twice as much as the inbreeding expected in an idealized population. In the absence of selection and under random mating, the expected level of inbreeding per generation is 1.56%: $\Delta F = 1/8N_m + 1/8N_f$ (Falconer, 1986). The mass spawning breeding schemes in both hapas had ΔF values three times the inbreeding constraint of 1% per generation generally given for breeding programmes (Bijma, 2000). Small scale, mass-spawning based genetic improvement programmes commonly produce lager number of offspring from a limited number of parents and often produce their own broodstock replacements. This together with the high observed variation in reproductive success and dominance of few males will have serious genetic consequences and might affect the fitness of the population in subsequent generations.

In conclusion, this study represents the first step in understanding the mating patterns in *O. niloticus* to quantify variance in reproductive success and estimate N_e. Further research should be carried out to evaluate the influence of different factors affecting mating strategies (such as sex ratio, stocking density etc) and hence optimize N_e.

Acknowledgements

This study is part of the first author's PhD study, funded by NUFFIC (The Netherlands organization for international cooperation in higher education), INREF-Pond, Wageningen University (http://www.inref-pond.org) and the WorldFish Center. We acknowledge the staff of The WorldFish Centre, Abbassa, Egypt for providing the necessary help and facilities during the experiments.

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Chapter 3

Effects of inbreeding on survival, body weight and fluctuating asymmetry (FA) in Nile tilapia, *Oreochromis niloticus*

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Aquaculture (*accepted*)

Abstract

The present study investigated the effects of different levels of inbreeding on survival, growth, body weight and fluctuating asymmetry (FA) in *O. niloticus*. We mated 20 sires and 35 dams in a full-sib/half-sib mating design to produce 35 full-sib families with expected level of inbreeding (F %) of 0.0, 6.3, 9.4, 12.5 and 25. Fry were produced and reared in hapas suspended in fertilized ponds until time of tagging. In total 1832 fish were tagged with floy® tags and stocked in two fertilized earthen ponds. During the experiment fish were not fed but relied entirely on natural food available in the ponds. Fish were harvested after a period of 8 months, which included 3 months of over-wintering. Results show that level of inbreeding significantly affected early fry survival and body weight at stocking. Level of inbreeding did not affect survival and body weight at harvest. Pond, weight at stocking, sire and dam had significant effects on body weight and pond survival at harvest. FA was not significantly affected by the level of inbreeding or sire. However, FA was significantly affected by dam (which constitutes the combined effects of hapa rearing, age of fish and maternal (genetic) effects), and pond. This supports the idea that FA is more influenced by environmental than by genetic factors. The observed difference in effects of inbreeding on early and later life stages can be explained by strong natural selection on fitness during over-wintering.

1. Introduction

Harmful effects of inbreeding have been noticed in numerous animal and plant species for many centuries. The magnitude and specific effects of inbreeding, however, are highly variable because they depend on the genetic constitution of the species/populations under study, and on interaction of genotypes with environment (Hedrick and Kalinowski, 2000). Inbreeding depression studies in fish are limited to a few experiments, mostly on rainbow trout (Aulstad and Kittelsen, 1971; Kincaid, 1976a,b; Gjerde et al., 1983; Su et al., 1996; Pante et al., 2001). The estimates of these studies in fish came from analyses with different models, conducted at different time periods and on populations with dissimilar levels of average inbreeding. In general, they show the detrimental effects of inbreeding such as reduced growth, viability, survival and increased number of abnormalities.

Inbreeding depression is more often observed in life-history traits than in morphological traits (DeRose and Roff, 1999). However, several studies have also found evidence of inbreeding depression on developmental stability of morphological traits (Leary et al., 1983; Alibert et al., 1994). Inbreeding may affect developmental stability of morphological traits because the lack of enzymatic diversity in homozygotes reduces the efficiency of normal developmental homeostasis processes (Lerner, 1954: Quoted in Radwan and Drewniak, 2001), this being reflected by an increase in fluctuating asymmetry (FA: Palmer and Strobeck, 1986). FA refers to a pattern of bilateral variation in a sample of individuals, where the mean of right minus left values of a trait is zero and the variation is normally distributed about that mean (Palmer, 1994).

In a previous paper (Fessehaye et al., 2006) we reported on the mating patterns of Nile tilapia under semi-natural mating conditions. Natural reproduction in *O. niloticus* is characterised by a high reproductive capacity of females, skewed reproductive success of males and very high variance in reproductive success. These factors are likely to increase the level of inbreeding in small populations; we estimated that natural mating could lead to an inbreeding level twice as high as what is expected in an idealized random mating population of the same census size.

Although inbreeding has been shown to depress fitness in many species, there is little knowledge on how inbreeding is manifested in fish at different stages of life and to our knowledge no study has so far been conducted to examine the effect of inbreeding on life-history and morphological traits in Nile tilapia. In the present study, we conducted single pair matings between pedigreed fish to produce progeny groups of fish with different levels of inbreeding in order to investigate the consequences of inbreeding on body weight, growth, survival and fluctuating asymmetry in *O. niloticus*

2. Materials and methods

2.1. Study population

All experimental procedures were conducted at The WorldFish Regional Center experimental station at Abbassa, Egypt. The base population at the station was produced in the spring of 2000 from all possible diallele crosses between four local Egyptian strains (Rezk et al., 2002). Individuals in the base population (G_0) were unrelated. Under the selection programmes described in Charo-Karisa (2006), G_1 and G_2 populations were produced in 2002 and 2003 respectively. Pedigreed and non-inbred fish from the second generation (G_2) of this selection programmes were used to produce offspring with five levels of inbreeding (F, %: 0, 6.3, 9.4, 12.5, and 25). Pedigree information was used to determine the level of inbreeding relative to the known founders of the population (G_0) which were assumed to be unrelated. We followed Wright's (1922) approach of path counting to calculate the additive genetic relationship between parents. Inbreeding was calculated as half the additive genetic relationship of the parents.

To disentangle the additive genetic effects and the effects of inbreeding, the experimental fish were produced in a full-sib/half-sib mating design in which each sire was mated to two dams and each dam mated to only one sire to produce half-sib families with different levels of inbreeding. E.g. a sire was mated to one dam giving progeny with expected inbreeding level of F = 25% and mated to another dam to produce progeny with F = 0%. In total 35 full-sib families were produced from 20 sires and 35 dams. These include 15 sires which were mated with two dams and five sires which were mated to one dam each.

Fry were produced over a period of five weeks (August 18 till September 22, 2004). At swim-up, each family was reduced to 100 fry per hapa (hereafter 'thinning') and separately reared in a 2 × 3 m hapa until the last family reached tagging size (mean of 2 g). Hapas were set in one 1000 m^2 earthen pond that received a daily application of chicken manure at the rate of 50 kg/ha/day. No supplemental feeds were given throughout the growth period.

At an average age of 8 weeks post hatching, a maximum of 60 fish/family were tagged with Floy® tags between the dorsal fin and lateral line. After tagging, fish were returned to hapas and allowed to recover from tagging stress for 4 days. The 60 progenies from each family were then divided into two random groups of 30 fry and each group was stocked in one of two 1000 m^2 grow-out ponds. In total 947 and 944 fish were stocked; 53 and 56 untagged fish were added to the first and second pond respectively to adjust the stocking density to 1 fish per m^2. Ponds were fertilized with 50 kg/ha/day of chicken manure on a daily basis. Fish were harvested after a period of about 8 months, which included an over wintering period of 3 months.

2.2. Data

Measurements in hapas (Hapa rearing)

At swim-up, the total number of hatched fry and the number of fry with deformities per dam were counted. Fry were again counted at the time of tagging and hapa survival (hereafter 'early fry survival') was calculated as $(N_t / N_0)x100$, where N_t is the number of fry at tagging and N_0 is the number of fry at thinning. At the time of stocking, fish were individually weighed.

Measurements in Ponds (Pond rearing)

Eight months after stocking, fish were harvested and the following measurements were taken: body weight, standard length, total length, sex, gonad weight and maturity stage of gonads. Total number of fish was counted to calculate survival of fish between stocking and harvesting (hereafter '*pond survival*'). Pond survival for each individual fish was recorded on binary scale (1 = survived, 0 = died). Maturity indices were recorded after visual examination of the gonad and evaluating the stage of development based on size, shape, texture and colour of the gonads as described in Owiti and Dadzie (1989). Maturity stages of 3, 4 and 5 were categorized as mature and scored as 1; maturity stages of 1 and 2 were categorized as immature and scored as 0.

Specific growth rate (SGR, % day^{-1}) was calculated as: $100x(\ln BW_f - \ln BW_i)/t$, where BW_i, BW_f are individual body weight at stocking and harvest respectively and *t* is the number of days between stocking and harvesting.

Gonado-somatic index (GSI): was calculated as $100x(Gonadweight/BW)$ where BW is the body weight of fish.

Fluctuating asymmetry (FA)

A population of 319 fish (Average weight = 156.77, CV = 30.8%) across different levels of inbreeding (see Table 7) were selected for the measurement of fluctuating asymmetry. Pectoral fin, pelvic fin and first gill branchial arches were dissected from both sides of the fish, cleaned and examined using a dissecting microscope. Four meristic traits (number of pectoral fin rays, number of pelvic fin rays, number of gill rakers on the upper first branchial arch and number of gill rakers on the lower first branchial arch) and one metric trait (head length) were measured on both the left and right side of all fish. Gill rakers were divided into upper and lower gill rakers because these have been show to vary independently (Leary et al. 1983). All measurements were taken by one observer to ensure consistent measurements on all fish.

2.3. Data Analysis

Hapa rearing

To analyze the factors affecting early life traits, two models were used. Model 1 was used for those traits with only one record per dam. Model 2 was used for traits with several records per dam

Total number of hatched fry, hapa survival, and fry deformity

The effects of dam weight and inbreeding level of offspring on total number of hatched fry, hapa survival and fry deformity were evaluated using the following model:

$$Y_i = \mu + \beta_1 * ln(W_i) + \beta_2 F_i + \varepsilon_i \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(Model 1)}$$

Where Y_i is the trait measurement on offspring of i^{th} dam; μ is the overall mean; $ln(W_i)$ is the co-variable of the natural logarithm of body weight of i^{th} dam; β_1 is the regression coefficient of Y_i on $\ln(W_i)$; F_i is the inbreeding level of the offspring produced by i^{th} dam; β_2 is the regression coefficient of Y_i on F_i; and ε_i is the residual error term.

Weight at stocking

The effects of dam, sire, level of inbreeding on weight of individual fish at stocking was tested using the following model:

$$Y_{ijk} = \mu + S_i + D_j(S_i) + \beta F_{ijk} + \varepsilon_{ijk} \qquad \text{(Model 2)}$$

Where Y_{ijk} is the natural logarithm of the stocking weight of an individual fish; μ is the overall mean; F_{ijk} is the level of inbreeding of an individual fish; β is the regression coefficient of Y_{ijk} *on* F_{ijk}; S_i is the effect of the i^{th} sire; $D_j(S_i)$ is effect of the j^{th} dam nested within the i^{th} sire and ε_{ijk} is the residual error term.

Pond rearing

Body weight at harvest, SGR, and GSI

The effects of pond, sex, sire, dam nested with in a sire, level of inbreeding and stocking weight on body weight at harvest, SGR and GSI were tested using the following model

$$Y_{ijkl} = \mu + P_i + S_j + D_K(S_j) + SEX_l + \beta_1 * ln(W_{ijkl}) + \beta_2 F_{ijkl} + \varepsilon_{ijkl} \qquad \text{(Model 3)}$$

Where Y_{ijkl} is the trait under study; μ is the overall mean; P_i effect of pond (i = 1, 2); S_j the effect of j^{th} sire; $D_K(S_j)$ is the effect of the k^{th} dam nested within the j^{th} sire; SEX_l the effect of sex (l = male, female); $ln(W_{ijkl})$ is the co-variable of the natural logarithm of weight of fish at stocking; β_1 is the regression coefficient of $\ln(W_{ijkl})$; F_{ijkl} is the co-variable of the level of inbreeding; β_2 is the regression coefficient of the level of inbreeding and ε_{ijkl} is the residual error term.

Survival and maturity

Generalized linear model (GENMOD) procedure with binomial distribution and logistic link function (SAS, 1989) was used to analyze the relationship between the response variable (pond survival and maturity scores) and the different explanatory variables (level of inbreeding, natural logarithm of body weight at stocking, effect of dam and sire, pond etc).

Analysis of FA

Paired measurements of a trait were entered for each individual and transformed into signed value of the difference between the right and left sides (R - L). Several confounding factors can obscure or inflate estimates of FA, such as directional asymmetry and antisymmetry. To ensure that the asymmetry present is true FA, several steps, as described in Palmer and Strobeck (1986) were followed. The presence of directional asymmetry (normal distribution with a mean different from zero) was tested using one-sample *t*-test (H_0: mean = 0.0). The distribution of the unsigned asymmetry values for each character was also examined to detect antisymmetry (usually a bimodal distribution with a mean of zero) using *t*-test of skewness and kurtosis measures (Van Valen, 1962; Palmer, 1994). We tested for a relationship between the magnitude of FA and character size by regressing signed trait asymmetry (R-L) on average of trait size (R+L)/2.

A multi-trait asymmetry composite index $\sum_{i=1}^{i=N}(R_i - L_i) \Big/ N$ (Palmer and Strobeck, 1986) was used to compare overall FA variation between fish of different inbreeding levels. R_i- L_i is the right-left value for i^{th} trait and N is the number of traits exhibiting FA. A model analogous to Model 3 was used to analyze the effects of pond, sex, sire, dam nested within a sire, and level of inbreeding on the multi-trait composite index of FA. Analyses were performed using Proc GLM of SAS (1989).

3. Results

Hapa rearing

Total number of hatched fry, hapa survival, fry deformity, and weight at stocking

Weight of the dam, number of families stocked, total number of hatched fry, body weight at stocking and early fry survival is presented in Table 1. Table 2 shows the results of the GLM analyses (Model 1) for total number of hatched fry, fry deformity and hapa survival. Total number of hatched fry was negatively affected by the level of inbreeding ($\beta = -9.59$; $P = 0.07$) but the weight of the dam had a positive effect ($\beta = 339.23$; $P = 0.08$). Level of inbreeding had a negative effect on early fry survival ($\beta = -0.55$; $P = 0.06$); weight of the dam did not have any effect on fry survival. The percentages of deformed fry were 8.27% and 3.26% for two families with inbreeding levels of F = 12.5% and F = 25% respectively. However, level of inbreeding and weight of the dam did not affect the proportion of deformed fry.

Table 1. Mean and standard deviation (in brackets) of body weight of dam (W_D), total number of hatched fry per dam, body weight of fry at stocking (W_S) and hapa survival for different levels of inbreeding.

W_D (g)	F (%)*	No. of families	No. of hatched fry	W_S (g)	Hapa survival
90.35 (27.25)	0.0	6	474.0 (217.2)	3.39 (4.06)	70.5 (24.30)
81.34 (23.43)	6.3	7	385.0 (213.5)	1.81 (2.60)	67.12 (21.51)
79.75 (21.20)	9.4	4	343.8 (387.0)	2.27 (1.19)	67.77 (6.57)
94.10 (13.59)	12.5	9	507.1 (354.9)	1.58 (0.70)	59.85 (2.57)
86.44 (16.02)	25.0	8	197.3 (110.8)	2.47 (1.39)	57.53 (6.90)

*F (%) is the expected level of inbreeding in progeny

Table 2. Marginal (type III) mean squares and *P*-values of level of inbreeding and body weight of dam on total number of hatched fry, deformity and hapa survival.

Source	*df*	Tot. hatched fry		Deformity		Hapa survival	
		MS	*P value*	MS	*P value*	MS	*P value*
F (%)	1	236317	0.07	10.73	0.85	779.47	0.06
Dam weight	1	207714	0.08	5.59	0.80	57.75	0.60

The GLM analysis (Model 2) as shown in Table 3 indicated that effect of level of inbreeding on body weight at stocking was highly significant ($\beta = -0.14$; $P < 0.001$). Both sire ($P < 0.001$) and dam components ($P < 0.001$) also had significant effects on the weight of fish at stocking.

Table 3. Marginal (type III) mean squares and *P*- values of the effects of sire, dam and level of inbreeding on log- transformed weight at stocking.

Effects	*df*	Mean square	*P* value
F (%)	1	5.542	< 0.001
Sire	19	13.715	< 0.001
Dam (Sire)	15	10.514	< 0.001

Pond rearing

Body weight at harvest, SGR, and GSI

Descriptive statistics on final body weight at harvest and pond survival are shown in Table 4. The results of the GLM analyses (Model 3) for different traits are shown on Table 5. There were significant effects of pond, sex, stocking weight, sire and dam ($P < 0.001$) on the log-transformed final body weight of fish at harvest. Specific growth rate of fish was significantly affected by pond, sex, log-transformed body weight at stocking, sire and dam ($P < 0.001$). Gonado-somatic index (GSI) was significantly affected by sex ($P < 0.001$), stocking weight ($P = 0.010$) and sire ($P = 0.002$). The level of inbreeding did not have significant effect on body weight at harvest, specific growth rate and Gonado-somatic index.

Table 4. Numbers of families and fish at stocking and harvest, and mean (standard deviation) of pond survival and body weight at harvest of fish for different levels of inbreeding.

F	Stocking		Harvest			
(%)	No. of families	No. of fish	No. of families	No. of fish	Survival	Weight
0.0	6	372	6	80	24.52 (16.50)	159.73 (48.58)
6.3	7	364	5	70	22.83 (15.85)	155.22 (44.36)
9.4	4	233	4	69	27.13 (18.70)	153.92 (43.46)
12.5	9	476	8	62	12.88 (14.82)	146.78 (48.12)
25.0	8	387	8	108	23.57 (16.19)	156.20 (51.41)

Pond survival and maturity

The results of the GENMOD analysis of factors affecting survival and maturity are show in Table 6. Pond survival of fish was significantly affected by sire, dam, natural logarithm of stocking body weight and pond (P < 0.001; χ^2 values of 453.32, 35.74, 40.17 and 82.82 respectively). The effect of inbreeding on survival was not significant ($P = 0.140$). Maturity was significantly affected by sire ($P = 0.015$), dam ($P = 0.008$), and stocking weight ($P = 0.018$). The level of inbreeding, sex and pond did not significantly affect maturity.

Table 5. Marginal (type III) mean squares and *P* values of the effects of different factors on log-transformed body weight at harvest (LnW$_f$), Specific growth rate (SGR), and Gonado-somatic index (GSI).

Source	*df*	LnW$_f$		SGR		GSI	
		MS	*P value*	MS	*P value*	MS	*P value*
pond	1	2.19	< 0.001	4.03 $\times 10^{-5}$	< 0.001	1.23	0.27
Sex	1	4.13	< 0.001	4.60 $\times 10^{-5}$	< 0.001	34.78	< 0.001
Ln(Wi)	1	1.56	< 0.001	26.63 $\times 10^{-5}$	< 0.001	6.80	0.010
Sire	18	0.29	< 0.001	0.53 $\times 10^{-5}$	< 0.001	2.36	0.002
Dam (Sire)	14	0.53	< 0.001	0.98 $\times 10^{-5}$	< 0.001	0.86	0.59
F (%)	1	0.09	0.23	0.18 $\times 10^{-5}$	0.23	0.69	0.41

Table 6. General linear model analysis (Genmod) of the effects of different factors on survival and sexual maturity of fish

Effects	Survival			Maturity		
	Df	χ^2	*P-value*	Df	χ^2	*P-value*
Sire	19	453.32	< 0.001	17	31.4	0.015
Dam (Sire)	14	35.74	0.001	13	28.36	0.008
F (%)	1	2.17	0.140	1	0.02	0.882
Ln(Wi)	1	40.17	< 0.001	1	5.56	0.018
pond	1	82.82	< 0.001	1	0.36	0.550
Sex	-	-	-*	1	0.06	0.801

* Not included in the model

Models

To examine if the dam effect in the model absorbs some of the effects of inbreeding, we run models 2 and 3 with and without the dam effect. Including the dam effect in the model increased both the parameter estimate of effect of inbreeding and the r-square (r^2) values and decreased the residual variance of the model. Both linear and quadratic terms of the level of inbreeding (F %) were tested for all the models and traits studied. No significant quadratic effect of inbreeding level was found for any traits. All the models were, therefore, run with the dam effect included and with inbreeding level as linear term.

Fluctuating asymmetry

Regression analysis of signed asymmetry on average trait size (analysis not shown) indicated that there was no significant dependence of the difference between sides (R-L) on average trait size (R+L)/2. Due to the absence of evidence for significant positive size dependence, size scaling of the FA traits was not necessary. The signed and untransformed difference between left and right trait values (L-R) was used for FA analyses.

Summary of asymmetry characteristics of five traits for different levels of inbreeding is shown Table 7. A *t*- test showed that the means of most of the asymmetry distributions did not significantly differ from zero except pectoral and pelvic fin rays ($P > 0.05$). Most of the traits, therefore, did not show directional asymmetry. The means of the asymmetry distributions of the number of pectoral fin rays (F = 0.0, 6.3%) and pelvic fin rays (F = 6.3%) significantly differed from zero ($P < 0.05$) suggesting the presence of directional asymmetry in those traits. The Shapiro-Wilk test showed that most of the asymmetry distributions were not normally distributed ($P < 0.05$). Test for kurtosis showed that most of the asymmetry distributions were leptokurtic indicating absence of antisymmetry. Whenever individual differences in developmental imprecision exists, FA should be leptokurtically distributed (Leung and Forbes, 1997; Gangestad and Thornhill, 1999). The tests above showed that most of the traits did not show directional asymmetry or antisymmetry. Bilateral variations were, therefore, mostly the consequence of FA.

The GLM analysis of factors affecting the composite FA index is shown in Table 8. The degree of asymmetry was not significantly affected by the level of inbreeding ($P = 0.10$), sex and sire component. Composite FA index was significant affected by Pond ($P = 0.004$) and dam ($P = 0.03$).

Table 7. Summary of asymmetry characteristics of five traits for different levels of inbreeding for *O. niloticus*

F (%)	0.0	6.3	9.4	12.5	25
Characters	(n = 60)	(n = 49)	(n = 58)	(n = 44)	(n = 108)
No. of pectoral fin rays					
Mean (SE) of R-L	0.60 (0.32)	0.81 (0.31)	-0.16 (0.31)	-0.28 (0.34)	0.15 (0.23)
t –test	*	*	NS	NS	NS
Shapiro –Wilk test	NS	*	*	NS	**
Kurtosis	P	Y	Y	P	Y
Type of asymmetry	DA	DA	FA	DA	FA
No. of pelvic fin rays					
Mean (SE) of R-L	0.17 (0.25)	0.63 (0.30)	0.30 (0.32)	0.14 (0.28)	0.11 (0.23)
t –test	NS	*	NS	NS	NS
Shapiro –Wilk test	*	*	*	NS	**
Kurtosis	Y	Y	Y	Y	Y
Type of asymmetry	FA	DA	FA	FA	FA
No. of upper gill rakers					
Mean (SE) of R-L	-0.10 (0.16)	0.10 (0.18)	-0.05 (0.19)	-0.25 (0.21)	-0.16 (0.11)
t –test	NS	NS	NS	NS	NS
Shapiro –Wilk test	**	**	**	*	**
Kurtosis	Y	P	Y	P	Y
Type of asymmetry	FA	FA	FA	FA	FA
No. of lower gill rakers					
Mean (SE) of R-L	-0.15 (0.22)	-0.42 (0.22)	-0.48 (0.29)	-0.77 (0.33)	-0.10 (0.16)
t –test	NS	NS	NS	NS	NS
Shapiro –Wilk test	NS	**	**	**	**
Kurtosis	Y	Y	Y	Y	Y
Type of asymmetry	FA	FA	FA	FA	FA
Head length					
Mean (SE) of R-L	0.011 (0.012)	0.03 (0.011)	0.01 (0.011)	0.05 (0.025)	0.05 (0.010)
t –test	NS	NS	NS	NS	NS
Shapiro –Wilk test	**	**	**	**	**
Kurtosis	Y	Y	Y	Y	Y
Type of asymmetry	FA	FA	FA	FA	FA
Composite FA index[a]	-0.018	-0.095	-0.076	-0.21	0.01

t –test → H_0: $\mu = 0.0$

* $P < 0.05$; ** $P < 0.01$; NS = not significant

P = Platykurtic; Y = Leptokurtic; FA = Fluctuating Asymmetry; DA =Directional Asymmetry

[a] Composite FA index is calculated based on traits exhibiting FA

Table 8. Marginal (type III) mean square and *p* value of the effects of different factors on the composite FA index

Source	*df*	Mean square	*P* value
sire	17	12.67	0.61
Dam(sire)	11	30.91	0.03
Sex	1	0.03	0.95
Pond	1	128.30	0.004
F (%)	1	39.41	0.10

4. Discussion

Inbreeding in early stage of development

Inbreeding can bring deleterious recessive alleles together in homozygous genotypes, thereby exposing them to greater selective forces and hence leading to reduced fitness known as ‘inbreeding depression’ (Crnokrak and Roff, 1999). In the present study, the significant effects of inbreeding on early life traits such as total number of hatched fry, early fry survival and body weight at stocking is consistent with many other studies that observed inbreeding depression in traits of early fitness (e.g. juvenile survival). Longwell and Stiles (1973) reported that progeny from full-sib matings of the American oyster (*Crassostrea virginica*) produced significantly lower survival of larvae to metamorphosis. Mrakovcic and Haley (1979) reported significant effect of inbreeding on survival to 30 days and length at day 30 in Zebra fish (*Brachydanio rerio*). Gjerde et al. (1983) studied the effect of three levels of inbreeding (F = 25, 37.5 and 50%) on the performance of *Salmo gairdneri* and observed that inbreeding depression for survival of eyed eggs, alevins and fry was highly significant for each level of inbreeding. Evans et al. (2004) found that individual growth and survival of *Crassostrea gigas* was significant depressed in families with F = 20.3%, but not in families with F = 6.3%. In our study, the relationship between effects of inbreeding and level of inbreeding is nearly linear for both early fry survival and body weight at stocking. A linear decline in the mean value of body weight with an increase in inbreeding level has also been observed in rainbow trout (Kincaid, 1976a,b, 1983; Su et al., 1996; Pante et al., 2001). However, the relationship between survival and level of inbreeding in rainbow trout appeared to be non-linear at an early stage (Kincaid, 1976a,b; Gjerde et al., 1983).

Wang et al. (2002) attributed this poor fit to either incomplete survival data or use of inappropriate model to analyze the data.

Inbreeding in later stages of development

Inbreeding did not have significant effects on body weight and survival at later stages of life. This is in agreement with results from Aulstad et al. (1972) who reported non-significant effect of inbreeding depression (F = 25%) for growth of rainbow trout fry at 331 days. Gjerde et al. (1983) also reported significant effect of inbreeding on survival of rainbow trout fingerlings, while fry weight at 160 days after first feeding was not significantly affected by any of the inbreeding levels studied (F = 25, 37.5 and 50%). With inbreeding, recessive alleles conferring inbreeding depression are exposed to natural selection, and therefore may be purged. As a result, inbreeding depression is expected to be reduced over time in groups of older individuals (Lande and Schemske, 1985): when inbreeding is high, lethals and sublethals expressed early in development leave relatively more heterozygous individuals and thus give lower estimates of inbreeding depression at later stages. Organisms with high fecundity most easily absorb (and hide) such inbreeding depression in later stages of life (Savolainen and Hedrick, 1995). However, Kincaid (1983) and Su et al. (1996) reported that the effect of inbreeding increased with age on traits such as body weight. This trend of increasing depression in body weight with fish age might be associated with the cubic nature of the growth curve magnifying the reduced growth rate of the inbred fish (Kincaid, 1983). However, even when inbreeding depression in body weight at the various age stages was calculated in % of the mean in an attempt to eliminate the effect of scale, the overall trend was still there (Su et al., 1996). It is possible that the lower survival at later stages of development in our experiment might be responsible for this difference in observations between trout and tilapia. Over-wintering survival in our experiment was very low for all levels of inbreeding (≈ 20%) and survival at harvest was significantly affected by body weight of fish at stocking (Table 6). Charo-Karisa et al. (2005) showed that smaller fish in *O. niloticus* are more susceptible to low temperature stress. Lower temperature during over-wintering, therefore, might have killed smaller individuals across all inbreeding levels. Another important difference between our study and that of Kincaid et al. (1983) is that in our study, fish were reared under semi-natural conditions, i.e. without any additional feeding. The apparent lack of inbreeding depression in later stages of life could, therefore, be due to the combined severe selective forces of low food availability and low temperature during over-wintering period, which might have diminished the differences in survival among the different levels of inbreeding and hence might have masked the effect of inbreeding.

Fluctuating asymmetry

Developmental stability is thought to depend upon heterozygosity. Heterozygotes are able to synthesise a wider range of biochemical products and thus to adjust development to a broader range of environmental variables (Lerner, 1954). There is increasing evidence that environmental stress may increase FA (Parsons, 1990) but there are only few studies that clearly demonstrate the influence of genetic stress on developmental stability (Batter-ham et al., 1996; Brakefield and Breuker, 1996). Our results show that fluctuating asymmetry, as estimated by the composite FA index, was not significantly affected by the level of inbreeding. This is in agreement with Bongers et al. (1997) who compared three F-isogenic and one partly outbred strains of carp, and concluded that the level of inbreeding does not seem to affect the true environmental variation and developmental stability. The present study also shows that no effect of sire was found while the dam and pond effects were highly significant. The dam effect, in our experiment, constitutes the effect of hapa rearing, maternal (genetic) effects and the age of fish. These results suggest that the contribution of genetic factors to variation in FA is limited. Genetic variation for FA is often assumed to be maintained by heterosis, on the basis that heterozygotes for enzyme–coding loci will be better able to withstand stress imposed by variable environments (Lerner, 1954: Quoted in Radwan and Drewniak, 2001). However, in recent years, the presumed link between allozyme/protein heterozygosity and FA has been increasingly questioned (Clarke 1993). A meta-analysis by VØllestad et al. (1999) showed that heterozygosity explained only a very small (1%) amount of variation in FA, and most investigators now appear to agree that there is very little additive genetic variation for FA in most characters. Our results support this conclusion.

Concluding remarks

The results of this study emphasize that inbreeding can have significant effects on early life traits such as fry survival and body weight, but not on later stages of development. The underlying reason for this could be that the set of detrimental alleles primarily responsible for lowered fitness due to inbreeding is effectively purged by (natural) selection. However, reduced differences between inbred and outbred individuals within small populations should not be used to infer that purging has been effective in eliminating all the negative effects of inbreeding as inbred individuals that survive to adulthood may still suffer reduced fitness via poor performance in mating competition and reduced fecundity.

Acknowledgements

This study is part of the first author's PhD study, funded by NUFFIC (The Netherlands organization for international cooperation in higher education), INREF-Pond, Wageningen University (http://www.inref-pond.org) and the WorldFish Center. We acknowledge the staff of The WorldFish Centre, Abbassa, Egypt for providing the necessary help and facilities during the experiments.

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Chapter 4

Effects of inbreeding and sex ratio on reproductive success in mass-spawning of Nile tilapia, *Oreochromis niloticus*

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Abstract

This study investigated the effects of inbreeding on the mating system and reproductive success in Nile tilapia, *O. niloticus* under semi-natural conditions in breeding hapas. Two hapas were stocked with 36 fish at a female to male sex ratios of either 1 (hapa A) or 3 (hapa B). Fish used for this experiment were randomly taken from nine families (four fish per family) with different levels of inbreeding (0, 6.3, 9.4 and 25%). Fry were collected from the mouth of incubating females on a weekly basis and parentage assignment was done on 1120 offspring from 56 spawnings using seven polymorphic microsatellite markers. Results show that female reproductive output (fecundity), expressed as the total number of eggs per spawning, was significantly affected by body weight and level of inbreeding. Per 10% increase of the level of inbreeding, egg number declined by 11% of the mean. Male reproductive success, calculated as the proportion of offspring sired per spawning, was affected by the level of inbreeding, condition factor, gonad weight of males and sex ratio. Per 10% increase in the level of inbreeding, male reproductive success declined by 40%. The decline in reproductive success was higher in sex ratio 1 compared to sex ratio 3 indicating that the effects of inbreeding were magnified under higher male-male competition. Degree of relatedness between mating pairs (Kinship coefficient) did not have any effect on reproductive success of males. Overall, our findings show that inbred individuals have lower reproductive success and uncover a natural selective pressure that favors outbred males and that counterbalances accumulation of inbreeding

1. Introduction

Reproductive success in many animals has been shown to be influenced by, among other things, the level of inbreeding, sex ratio and genetic compatibility of potential mates. Inbreeding has been observed to cause differential mate acquisition and reproductive success in many vertebrate species (e.g. Meagher et al., 2000; Slate et al., 2000; Charpentier et al., 2005). Inbred adults may be at a disadvantage in direct intrasexual competition over mates because they are less vigorous than their outbred counterparts (Ryan et al., 2002); For example, inbreeding was demonstrated to reduce competitive male mating ability in *Drosophila melanogaster* (Miller et al., 1993) and to decrease display rate in guppies which is a primary determinant of reproductive success (Farr, 1983).

There is a growing body of evidence that genetic compatibility may drive mate choice and hence affect reproductive success (Tregenza and Wedell, 2000). The recognition and avoidance of kin as potential mate could affect mate acquisition and reproductive success in systems where individuals actively avoid inbreeding by mating with least-related mates available (Ryan et al., 2002). In many species, genetic similarity to females was found to affect male reproductive success: males genetically most different from females sired more offspring in multiply sired litters, despite having no mating advantage (Olsson et al., 1996). Among insects, Wilson et al. (1997) found that female genotype strongly influences the outcome of sperm competition in *Callosobruchus maculatus* with male success apparently determined by genetic compatibility with the female.

Reproductive success can also be influenced by a variety of ecological and demographic variables such as sex ratio (Emlen and Oring, 1977) and population density (Maher and Lott, 2000). Both sex ratio and density may influence the encounter rates between males and females and the extent to which mates can be monopolized (Emlen and Oring, 1977). Classical sexual selection theory predicts that the operational sex ratio (the ratio of sexually active males to fertilisable females) seem to trigger changes in competition intensity (Emlen and Oring, 1977; Reynolds, 1996), male mating tactics (Krupa and Sih, 1993) and reproductive success (Andersson, 1994; Reynolds, 1996).

In *Oreochromis niloticus*, territorial males provide no parental care and their mating system resembles that of lekking animals. In a lek mating system, males defend clustered territories and females visit males solely for courtship and mating (Bradbury and Gibson, 1983). In mass spawning in Nile tilapia, aggregation of reproductive individuals could provide us with interesting models to study the factors influencing mating systems and reproductive success under semi-natural conditions. In a previous paper (Fessehaye et al., 2006), we reported on the mating patterns of Nile tilapia under semi-natural mating conditions at a female to male sex ratio of 2. In the present study, we investigated the effects of inbreeding on the reproductive success in Nile tilapia. Fish were stocked at sex ratios of 1 and 3 to evaluate the effect of number of potential mates on reproductive strategies and mating systems. To test if the degree of relatedness between potential mates in a hapa influences the reproductive success, fish with different level of relatedness (kinship coefficient) were stocked.

2. Materials and Methods

Fish

The spawning experiments were carried out at the experimental station of The WorldFish Center, Abbassa, Egypt. Parental fish for this experiment were produced in 2004 (See Fessehaye et al., 2006 for details) from pedigreed and unrelated fish of the second generation of a breeding programme (described in Charo-karisa et al., 2006). A total of 72 fish (27 males and 45 females) from nine full-sib families (8 fish/family) were used. Based on pedigree, fish had inbreeding levels (F, %) ranging from 0- 25% (i.e. F= 0, 6.3, 9.4 and 25%; See Table 1). Females and males were separately conditioned for two weeks prior to stocking in the breeding hapas. During this period, fish were fed with 3mm floating pellets (40% crude protein, Alexandria Oil and Soap Company, Kafr El-Sheikh, Egypt) twice daily at of 3% body weight per day.

Experimental set up

The spawning experiments were carried out during the breeding season of 2005. Prior to stocking, all parental fish were measured for total body weight (W, nearest 0.1 g); total length (TL), and standard length (SL). TL and SL were measured as the distance from the tip of the snout to the end of the caudal fin and hypural bone respectively. For DNA analysis, 0.5 ml of blood was collected from all parental fish by caudal puncture using a hypodermic needle rinsed with Na_2EDTA. Two 8 x 2 x 1 m^3 (length x width x depth) hapa enclosures were installed in a concrete- walled pond with mud bottom (dimension: 50 x 5 x 1 m^3; the floor of the hapas was sitting on the pond bottom). Broodstock were divided into two groups consisting of 36 fish in such a way that the nine families were represented equally in both hapas. Hapa A was stocked with 18 females and 18 males (2 females and 2 males per family) at a female to male sex ratio of 1:1 (Sex ratio 1). Hapa B was stocked with 27 females and 9 males (3 females and 1 male per family) at female: male sex ratio of 3:1 (Sex ratio 3). Each family was represented by four full-sib fish in each hapa. Two days after stocking, one male and one female died in hapa A and B respectively and were replaced with another fish from the same family; fish which died at a later stage of experiment were not replaced (n = 4). Broodstock were allowed to spawn naturally for a period of ten days after which collection of eggs commenced.

Egg collection and incubation

Collection of eggs was carried out on a weekly basis for a duration of eight weeks. All females were checked during each collection period. Females carrying eggs in the buccal cavity were identified with the tag number and eggs were collected from the mouth of each incubating female by rinsing them into a plastic bowl. The fertilized eggs were counted and were incubated in 20-l aquaria with continuous aeration to keep the eggs in constant motion and to ensure sufficient oxygen supply needed for hatching of eggs. To control fungal infection, the water was treated with 30 ppm formalin for the first day and with 20 ppm formalin for the subsequent days. Formalin treatments were stopped when the embryos reached the eyed stage. Eggs were inspected daily and unfertilized or dead eggs were removed as the presence of spoiled eggs may cause infection and loss of the whole spawn. Embryos were reared in the incubation system till the yolk sac stage. Twenty yolk sac fry per spawn were randomly sampled for DNA extraction and genetic analysis.

DNA extraction and microsatellite analysis

Genomic DNA was extracted from blood of parental fish and from whole yolk sac larvae. DNA was isolated from blood of parental fish using the PUREGENE kit (Gentra Systems, Minneapolis, MN, USA) following the manufacturers instructions for non-mammalian blood. Yolk sac fry were overdosed with MS-222 (tricaine methanesulfonate). The yolk sac was then removed using a scalpel and embryos were placed in sterile 1.5 ml microcentrifuge tubes. The tissue was squashed and homogenized using micro a pestle and extraction of DNA from whole larvae was carried out using the same kit and protocol as for non-mammalian blood. The amount of re-hydrated DNA solution was adjusted to yield final DNA concentrations of 5-10 µg/ml.

Based on the parentage analysis in Fessehaye et al. (2006) and simulation results, seven polymorphic and informative markers (*UNH169, UNH190, UNH123, UNH178, UNH208, UNH214, UNH231*) were selected for genotyping all parental fish and offspring. PCR cycling conditions were as follows: 5 min at 95 °C, 35 cycles of 30 s at annealing temperature (45–60 °C), and 30s at 72 °C, followed by a final elongation step of 4 min at 72 °C. Amplified products of all markers were multiplexed in one set, and electrophoresed through a system of capillaries on ABI 3730 DNA analyzer (Applied Biosystems, CA, USA). Fragment sizes were calculated relative to the LIZ 500 size standard. Genotype results were analyzed using GeneMapper Version 4.0 (Perkin-Elmer, Boston, MA, USA).

Calculations and statistical analysis

Parameters calculated

Condition factor: Fulton's condition factor (K) was calculated as $k = BW/SL^3$, where BW is body weight (g) and SL is standard body length (cm).

Spawn fecundity (*number of eggs/spawning*) of females was equal to the total number of eggs counted per spawning.

Gonado-somatic index (GSI): At the end of the experiment, all parental fish were sacrificed and gonads weighed. GSI was calculated as $GSI = 100x(Gonadweight/BW)$ where BW is the live body weight of fish

Kinship coefficient (Φ): The kinship coefficient is half the additive genetic relationship of two individuals. The additive genetic relationship between two individuals was calculated as

$$a_{x,y} = \sum_{i=1}^{n} \left(\frac{1}{2}\right)^{(ni+pi)} (1+Fw_i)$$

Where $a_{x,y}$ is the additive genetic relationship between *x* and *y*; the Σ indicates the summation over all n paths connecting *x* and *y*; the subscript *i* indicates one specific path with (n_i + p_i) generations separating x and y via common ancestor W_i; Fw_i is the inbreeding coefficient of common ancestor W_i; n is the number of generations between *x* and W_i; p is the number of generations between *y* and W_i. Relationships during two generations were used to calculate the kinship coefficient relative to the known founders of the population (G_0) which were assumed to be unrelated and with F = 0. We followed Wright's (1922) approach of path counting to calculate the additive genetic relationship and F.

Genetically effective paternity frequency (PF) was calculated for each spawning following Bekkevold et al. (2002)

$$PF = 1 \Big/ \sum_{i=1}^{n} p_i^{\ 2} ,$$

Where p_i is the proportion of offspring sired by the i^{th} male, n number of males involved in fertilizing a spawn.

Parentage analysis

Allocation of offspring to their putative parental pairs was performed using the program PAPA ver. 2.0 (Package for Analysis of Parental Allocation) with a uniform distribution error rate of 0.02 (Duchesne et al., 2002). To test the reliability of parental assignment, 10,000 offspring (100 iterations and 100 offspring/iteration) were simulated based on the observed genotypes of all parents. Simulated offspring were re-allocated to their putative parents. The simulation was used to test the efficiency and reliability of the loci used in parentage assignments.

Reproductive success of females and males

The effect of inbreeding and body weight on spawn fecundity and gonad weight of females was analyzed using model 1. The same model was used to analyze effects of inbreeding and body weight on gonad weight of males:

$$Y_i = \mu + \beta_1 F_i + \beta_2 ln(wt_i) + \varepsilon_i \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(Model 1)}$$

Where Y_i is the trait under study; μ is the overall mean; F is the co-variable of level of inbreeding of individual fish; β_1 is the regression coefficient of F on Y_i; $ln(wt_i)$ is the co-variable of natural logarithm of the body weight of fish; β_2 is the regression coefficient of $ln(wt_i)$ on Y_i and ε_i is the residual error term.

Male reproductive success was calculated for each male as the proportion of offspring sired by that male in a spawning. The effects of sex ratio, inbreeding coefficient, condition factor of males, GSI of males, and kinship coefficient of mating pairs (male-female relatedness) on reproductive success of males were investigated by generalized linear model (GENMOD). A logistic regression with a binomial error distribution and logit-link function was used as male reproductive success was calculated as the proportion of offspring sired within a single spawning. Since level of inbreeding of males might be related to their condition factor and GSI, these factors were alternatively added to the basic model which includes effects of sex ratio and kinship coefficient. The model fitted was

$$E\ (RS) = \mu + Sexratio_i + \beta_1\Phi_j + \varepsilon_{ij} \ \ldots\ldots\ldots\ldots\ldots\ldots \text{(Model 2)}$$

Where *RS* is reproductive success of males; μ is the overall mean; *Sexratio* is the female to male sex ratio (*1, 3*); Φ_j is the co-variable of the kinship coefficient of the mating pairs; β_1 is the regression coefficient of the kinship coefficient. The variables alternatively added to the basic model: F_k is co-variable of the inbreeding level of the k^{th} male; β_2 is the regression coefficient of *F*; K_l is the co-variable of condition factor of the l^{th} males; β_3 is the regression coefficient of the condition factor of males (*K*); GSI is the co-variable of the gonado-somatic index of males; β_4 is the regression coefficient of GSI and ε_{ij} is the residual error term.

3. Results

Spawning, fecundity and gonad weight

Descriptive statistics on body weight, GSI (for both males and females) and total number of spawnings and spawn fecundity (females) for fish of different levels of inbreeding and for the two sex ratios are given in Table 1. The total number of spawns collected over the experimental period was 23 and 41 for sex ratios of 1 and 3 respectively. In the hapa with sex ratio of 1, 67% of the females spawned and about 39% of the females had repeated spawning. For sex ratio of 3, 78% of females spawned and 54% of the females spawned more than once. One spawn (sex ratio 1) and five spawns (sex ratio 3) did not show any sign of development and quickly succumbed to fungus, indicating that they probably had not been fertilized. Two batches of eggs in the sex ratio 3 hatched but did not develop into yolk sac fry. The overall mean (±SD) spawn fecundity was 849 (±537) and 562 (±286) for females in sex ratios of 1 and 3 respectively.

Table 1. Mean (standard deviation) of body weight, GSI, number of spawns collected and fecundity of fish used in the experiment

Level of inbreeding	Females					Males		
	No. stocked	Body weight	GSI	Total no. of spawning*	Spawn fecundity	No. stocked	Body weight	GSI
Sex ratio 1:1								
0	8	134.1 (38.8)	3.4 (2.4)	14 + [1]	948 (552)	8	169.8 (51.9)	1.4 (0.8)
6.3	2	128.6 (16.1)	2.4**	2	951(1133)	2	166.7 (39.0)	1.2 (0.13)
9.4	2	122.0 (13.2)	3.9**	1	469	2	109.2 (17.2)	0.16**
25	6	107.8 (24.9)	1.3 (0.3)	5	608 (238)	6	161.2 (35.6)	0.7 (0.4)
Sex ratio 3:1								
0	12	116.9 (32.7)	2.6 (2.0)	19 + [1]	529 (271)	4	164.0 (46.9)	0.8 (0.7)
6.3	3	118.3 (15.2)	1.4 (0.4)	5 + [3]	610 (374)	1	180.4	1.3
9.4	3	116.5 (14.0)	4.1 (1.4)	4 + [1]	576 (173)	1	119.2	0.6
25	9	113.6 (22.6)	2.2 (2.1)	6 + [2]	605 (357)	3	160.4 (51.6)	1.4**

*Numbers in brackets [ ] are number of spawns which did not hatch or spawns which hatched but did not develop to embryos

** SD could not be calculated because only one sample was available at the time of sampling

Results of analysis of factors affecting spawn fecundity of females (Model 1) are shown in Table 2. Female body weight had a positive effect on spawn fecundity (β = 706.6, *P*-value = 0.004). Level of inbreeding of the spawning female did not have significant effect on spawn fecundity (β = -7.3, *P*-value = 0.14). Table 3 depicts results of analysis of factors affecting the gonad weight of both females and males. Female gonad weight was negatively affected by the level of inbreeding (β = -0.05, *P*-value = 0.04) but body weight did not have significant effect on the gonad weight of females. Male gonad weight was positively affected by body weight (β = 1.92, *P*-value = 0.04) but level of inbreeding did not affect gonad weight in males.

Table 2. Marginal (Type III) mean squares and parameter estimates of the effects of female body weight and level of inbreeding (F, %) on the spawn fecundity of Nile tilapia females (Model 1)

Source	*df*	Mean Square	Parameter estimate	*P*-value
Ln (body weight)	1	1678904	706.6 (237.2)	0.004
Level of inbreeding	1	407194	-7.3 (4.9)	0.14
Error	67	12675852		

Table 3. Marginal (Type III) mean squares and parameter estimates of the effects of body weight (BW) and level of inbreeding (F, %) on the gonad weight of females and males (Model 1)

Source	Female				Male			
	df	Mean square	Parameter estimate	*P*-value	*df*	Mean square	Parameter estimate	*P*-value
Ln (BW)	1	1.29	-0.64 (1.10)	0.55	1	4.03	1.92 (0.87)	0.04
F (%)	1	15.69	-0.05 (0.02)	0.04	1	1.40	-0.03 (0.02)	0.21
Error	59	218.74			14	11.58		

Parentage analysis

Simulation results showed that 98.6% (sex ratio 1) and 98.3% (sex ratio 3) of the simulated offspring could be assigned to their putative parents based on the seven microsatellite markers used in this experiment. Parentage assignment of real collected offspring was performed on 440 (sex ratio = 1) and 680 (sex ratio = 3) offspring. Of the offspring analyzed, 97.5% and 98.8% were unambiguously allocated to their putative parents for sex ratio 1 and 3 respectively. Inspection of the genotypes of the offspring showed that 10 and 6 offspring in sex ratio 1 and 3 respectively did not resolve at most of the markers and were removed from the data set. After excluding those samples, about 99.8% (sex ratio 1) and 99.5% (sex ratio 3) of all offspring for both sex ratios could be unambiguously allocated to the putative parents. Cross checking of the custodian mothers and the genetic mothers showed that all the custodian mothers were the genetic mothers of most (> 90%) of the eggs they were incubating at the time of collection except for one female in sex ratio 3. The spawn collected from this female consisted of eggs which were exclusively spawned by other females.

Mating systems

Table 4 shows the prevalence of different mating systems in Nile tilapia observed for both sex ratios. About 14% and 32% of spawnings for sex ratio of 1 and 3 respectively involved single pair mating in which the eggs of one female were entirely fertilized by one male. Multiple paternity was observed in about 64% and 32% of the spawning in sex ratios of 1 and 3 respectively. In those spawnings, genotypes of up to five and three males were detected in a single spawn for sex ratios 1 and 3 respectively. Multiple maternity was detected in 2 spawnings (5.9%) in sex ratio of 3. No multiple maternity was observed in sex ratio of 1. About 23% (sex ratio 1) and 29% (sex ratio 3) of the spawnings had embryos from multiple mothers fertilized by multiple males (promiscuous matings).

Table 4. Effect of sex ratio on the mating patterns of Nile tilapia. Numbers in parentheses are percentage of the total spawns

Sex ratio	Single pair mating	Multiple paternity	Multiple maternity	Promiscuous matings	Total no. of spawnings
1:1	3 (13.6%)	14 (63.6%)	0 (0%)	5 (22.7%)	22
3:1	11 (32.4%)	11 (32.4%)	2 (5.9%)	10 (29.4%)	34

Male reproductive success

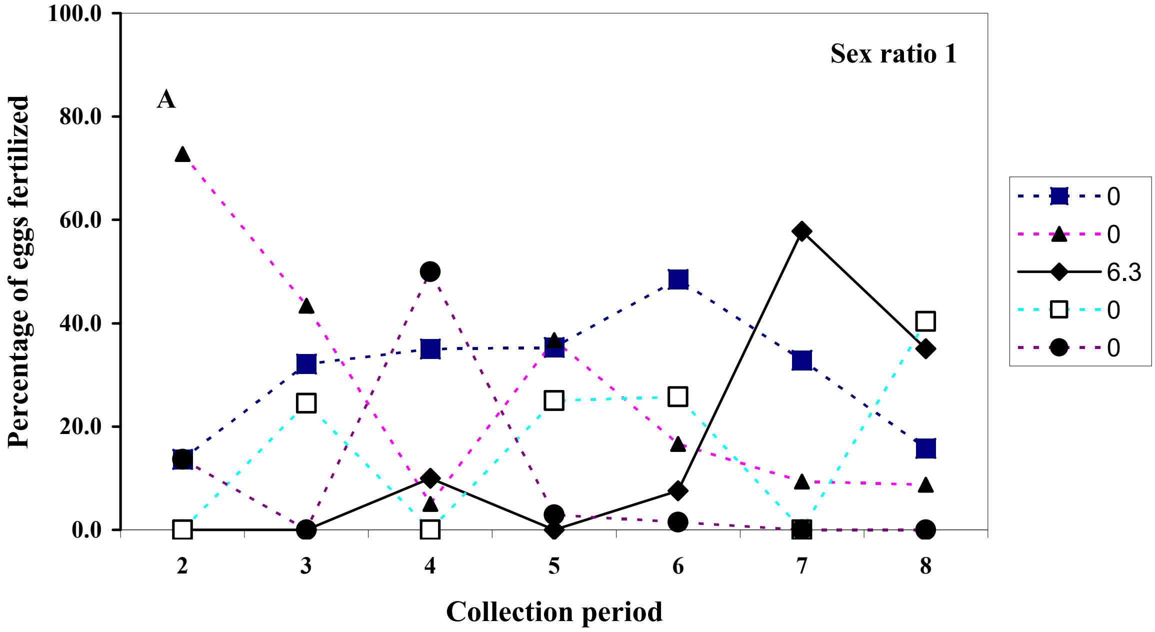
One third of the males sired about 89% and 93% of the analyzed offspring in sex ratio 1 and 3 respectively. Three males in sex ratio 1 and one male in sex ratio 3 did not sire any of the offspring analyzed. One male in sex ratio 1 died one day after stocking of fish and was excluded from the analysis. Promiscuous spawnings were regarded as reproductive artifacts and were not considered in further calculations and analysis. Excluding the promiscuous matings, the average number of fathers contributing to a spawning was 2.14 (SE = 0.41) and 1.18 (SE = 0.08) for sex ratios 1 and 3 respectively. The average proportions of offspring sired and the genetically effective paternity frequencies in spawnings with various numbers of males siring offspring are shown in Table 5. The mean genetically effective paternity frequencies for sex ratios 1 and 3 were 1.68 (SE = 0.24) and 1.20 (SE = 0.06) respectively. The ranking of males' paternity proportion given in Table 5 was further broken down into the relative contribution of the males with different categories of levels of inbreeding (Table 6). Males with lower inbreeding levels (0% and 6.3%) dominated the majority of the paternal proportions in each successive rank (from the most successful to the third most successful ranks).

Table 5. The average ± SD paternity proportions for spawnings in which from one up to five males participated. Male are ranked from most successful to least successful. The average genetically effective number of fathers is also given. Promiscuous matings were not included for calculation of effective paternity frequency (*n* = 5 and 10 for sex ratios 1 and 3 respectively)

No. of males participating in spawning	No. of spawnings (% of total)	Paternity proportion of most successful male	Paternity proportion of second most successful male	Paternity proportion of third-most successful male	Paternity proportion of the fourth-most successful mal	Paternity proportion of the least successful male	Genetically effective paternity frequency
Sex ratio 1:1							
1	3 (17.6%)	1.00 ± 0.0	-	-	-	-	1.00 ± 0.0
2	4 (23.5%)	0.86 ± 0.13	0.14 ± 0.13	-	-	-	1.30 ± 0.33
3	6 (35.3%)	0.70 ± 0.20	0.20 ± 0.10	0.10 ± 0.00	-	-	1.64 ± 0.36
4	2 (11.8%)	0.75 ± 0.0	0.14± 0.0	0.055 ± 0.0	0.055 ± 0.0	-	1.70 ± 0.14
5	2 (11.8%)	0.51 ± 0.30	0.16 ± 0.06	0.13 ± 0.10	0.10 ± 0.07	0.10 ± 0.07	3.50 ± 2.36
							1.68 ± 0.98[1]
Sex ratio 3:1							
1	13 (54.2%)	1 ± 0.0	-	-	-	-	1.00 ± 0.0
2	8 (33.3%)	0.86 ± 0.08	0.14 ± 0.08	-			1.32 ± 0.20
3	3 (12.5%)	0.73 ± 0.14	0.18 ± 0.13	0.09 ± 0.06	-	-	1.74± 0.45
							1.20 ± 0.31[1]

[1] overall average

The percentage of offspring sired by the five most successful males over the different collection periods is shown in Figure 1a (sex ratio 1) and 1b (Sex ratio 3). Both figures show that males with lower inbreeding levels were dominant over males with higher levels of inbreeding. At each collection period, there were more males participating in fertilization of eggs in the sex ratio 1 (average = 2.9, SD = 0.38) than in the sex ratio 3 (average = 2, SD = 1.1). These figures also show that there was a shift in reproductive dominance of males over the course of the spawning season.



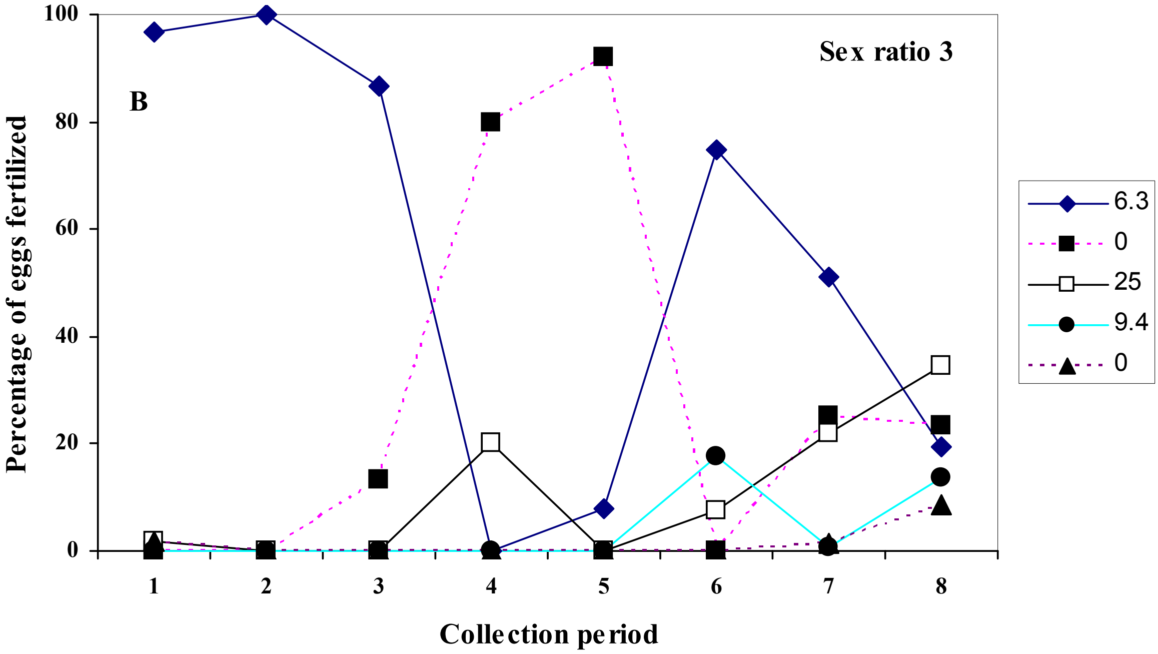


Figure 1a, b. Percentage of eggs fertilized by five most successful males at different collection periods for sex ratio 1 (A) and 3 (B). Legend shows the level of inbreeding for each male.

Table 6. The relative contribution of males of varying levels of inbreeding within each rank. Within each success rank contribution is broken down based on the levels of inbreeding of the contributing males

No. of males participating in spawning	No. of spawnings	Most successful males				Second most successful males				Third-most successful males			
		Level of inbreeding (%)				Level of inbreeding				Level of inbreeding			
Sex ratio 1:1		0	6.3	9.4	25	0	6.3	9.4	25	0	6.3	9.4	25
1	3	67%	33%	0	0	-	-	-	-	-	-	-	-
2	4	100%	0	0	0	90%	0	0	10	-	-	-	-
3	6	100%	0	0	0	84%	16%	0	0	43%	0	0	43%
4	2	48%	52%	0	0	40%	0	0	60%	100%	0	0	0
5	2	100%	0	0	0	100%	0	0	0	20	0	0	80%
Sex ratio 3:1													
1	13	23%	61%	8%	8%	-	-	-	-	-	-	-	-
2	8	48%	28%	11%	13%	0	40%	5%	55%	-	-	-	-
3	3	0	71%	0	29%	9%	55%	0	36%	100%	0	0	0

Results of the analysis of factors affecting reproductive success of males according model 2 are shown in Table 7. Male reproductive success was negatively affected by the level of inbreeding of males ($\beta = -0.04$, $P < 0.0001$) and sex ratio ($\beta = -0.74$, $P < 0.0001$), with males in the sex ratio 3 having higher reproductive success. Male condition factor ($\beta = 44.8$, $P < 0.0001$) and GSI ($\beta = 0.74$, $P < 0.0001$) had a positive effect on the reproductive success of males. The coefficient of kinship between mating pairs (male-female relatedness) did not have a significant effect on reproductive success ($\beta = -0.05$, $P = 0.89$). To test if the effect of inbreeding was different with different male density, the model was also run with the effect of inbreeding nested within sex ratio. The effect of inbreeding was higher in sex ratio 1 ($\beta = -0.089$, $P < 0.0001$) compared to sex ratio 3 ($\beta = -0.012$, $P = 0.008$) (analysis not shown).

Table 7. Generalized linear model (type III) chi-square values and parameter estimates of the effects of sex ratio, kinship coefficient, level of inbreeding, condition factor of males and GSI on reproductive success of males (Model 2)

Source	*df*	Chi-square	Parameter estimate	*P*-value
Sex ratio	1	135.63	-0.74 (0.06)	<0.0001
Kinship coefficient	1	0.02	-0.05 (0.39)	0.89
Level of inbreeding*	1	123.55	-0.04 (0.03)	<0.0001
Condition factor*	1	31.94	44.8 (9.60)	<0.0001
GSI*	1	143.66	0.74 (0.06)	<0.0001

* The factors in the basic model are sex ratio and kinship coefficient. Factors marked with asterisk (*) were alternatively added to the basic model.

4. Discussion

Mating systems

The mating systems observed in both sex ratios are diverse ranging from single pair mating to promiscuous mating. The frequency of single pair mating in sex ratio 3 (32%) is more than twice what is observed in sex ratio 1 (14%). Conversely, the frequency of multiple paternity in sex ratio 1 (64%) was twice that of the sex ratio of 3 (32%). The prevalence of monogamy is likely due to a combination of factors that reduce either the environmental potential for polygyny or the ability of males to exploit any existing polygyny potential (Emlen and Oring, 1977). Prevalence of single pair and multiple pair mating in spawning with sex ratio of 2 as reported in Fessehaye et al. (2006) was 21% and 46% respectively. These values are mid way in between the values of the sex ratios in the present study and thus reinforce our conclusion that differences due to sex ratio are causing differences in mating system in Nile tilapia. Over all, our result is in agreement with previous findings that adult sex ratio is a key factor affecting sexual competition (Kvarnemo et al., 1995) and mating system (Emlen and Oring, 1977).

Parental inbreeding and reproductive success

In the present study, a decline in egg number of 11% of the mean was observed per 10% increase of the level of inbreeding. Su et al. (1996) showed that the number of eggs in *Oncorhynchus mykiss* was significantly reduced by inbreeding (6% per 10% increase in inbreeding). The higher magnitude of inbreeding depression could be the result of fast accumulation of inbreeding by full-sib mating in our experiment as opposed to accumulation of inbreeding over five generations in Su et al. (1996). At a slow rate of inbreeding, there are more generations during which selection can counteract effects of inbreeding by eliminating a larger proportion of the genetic load (Ehiobu et al., 1989; Day et al., 2003; Reed et al., 2003).

Level of inbreeding had a significant effect on the reproductive success of Nile tilapia males. Per 10% increase in the level of inbreeding, the reproductive success of males decreased by 40% (Table 7). Visual inspection of multiply sired spawns also showed that relatively outbred males were responsible for fertilizing the majority of eggs while more inbred males had marginal contribution in 85% of the spawns. Although inbreeding has been shown to depress fitness traits in early stages of many species; there is little knowledge about the effects of inbreeding on reproductive success in fish in general and tilapia in particular. In a recent study of house mice (*Mus domesticus*), reproductive success of males was reduced by 81%

due to inbreeding (F = 0% vs. 25%) (Meagher et al., 2000). In mandrills (*Mandrillus sphinx*), less inbred males sired more offspring (Charpentier et al., 2005). In black grouse (*Tetrao tetrix*), males that were successful in securing a territory on a lek at least once in their life time had a higher heterozygosity than males that never obtained a territory (Höglund et al., 2002). Individual standard heterozygosity and reproductive success were positively correlated in red dear, particularly among males (Slate et al., 2000). Relative to more outbred control individuals, inbred *Mus domestsicus* males were less likely to gain territories. Territories are critical for male reproduction because in this species females mate almost exclusively with dominant, territorial males (Potts et al., 1994; Meagher, 2000). *O. niloticus* males are territorial and their mating system resembles that of other lekking animals. In the lek mating system males defend clustered territories and females visit males solely for courtship and mating (Bradbury and Gibson, 1983). The decline in reproductive success caused by inbreeding was higher for males in sex ratio 1 than sex ratio 3. This demonstrates that inbred males had lower reproductive success and the effect was magnified in the presence of higher number of males i.e. under more competitive conditions for males. This is consistent with other findings that inbreeding decreases a male’s success in aggressive encounters (Ekund, 1996) and its ability to obtain territories (Potts et al., 1994). Meagher et al. (2000) also reported that male-male competition magnified inbreeding depression in wild house mice.

Sex ratio and reproductive success

A Relatively higher frequency of female spawning was observed in the sex ratio with lower male density (sex ratio 3 = 78%) compared to the sex ratio with higher male density (sex ratio 1 = 67%). In other fish species, increase in male density has been shown to lead to increased aggression and male-male competition, resulting in reduced opportunities for females to spawn: e.g. Japanese medaka (Grant et al., 1995); European bitterling, *Rhodeus sericeus* (Reichard et al., 2004). We have no behavioural observation in the present experiment but higher aggression and male-male competition is expected in the high male density (sex ratio 1) and this might have affected female oviposition resulting in lower spawning frequency. This is in agreement with (Mills and Reynolds, 2003) who reported that at low male density, there is less aggression and more courtship by territorial males leading to increased female spawning frequency. On the other hand, at high male density there is break-down in territorial defense and courtship by dominant males. More courtship behaviour is observed at low density than at high density (Jirotkul, 1999; Kanoh, 2000) and at a low competitor-to-resource ratio (Grant et al., 2000). Five spawns in sex ratios of 3 and one spawn in and sex ratio 1 were not fertilized and succumbed to fungus. The lower male encounter together with increased courtship behaviour of males in lower male density in sex ratio 3 might have left some females with little mating opportunities resulting in their eggs to remain unfertilized. Reduction in the number of breeding males per female leads to the reduction in sperm concentration and can lead to reduced fertilization success, notably among organisms that spawn in aggregations and release their gametes directly in to the water (Marconato et al., 1997). Furthermore, two spawns in sex ratio 3 had genotypes from two females fertilized by a single male (multiple maternity) indicating some degree of female-female competition for male resources. Several earlier studies have observed that female-female competition increases in female-biased sex ratios (Grant and Foam, 2002; Debuse et al., 1999).

In the present study, males in sex ratio 3 had higher reproductive success compared to the males in sex ratio 1. This is not surprising and it simply could be the direct result of the presence of more females. As males obtain more mates, the average mating and reproductive success will also be higher. Many studies have shown that in male-biased sex ratios, males tend to compete more intensively for mates because they have more potential competitors (Emlen and Oring, 1977; Enders, 1993; Krupa and Sih, 1993). Even at unbiased sex ratios, males are expected to compete for higher female allocation. This idea is rooted in the assumption that males, but generally not females can potentially increase their reproductive success by increasing the number of mates (Ward and Fitzgerald, 1988). We have not directly observed male-male competition but higher occurrence of multiply sired spawns in sex ratio 1 (64%) as compared to 32% in sex ratio 3; and the higher average number of males fertilizing a spawn in sex ratio 1 (2.14 males) as opposed to 1.18 males in sex ratio 3 are indications that there was more male-male competition in sex ratio 1 compared to sex ratio 3. Figure 1a (sex ratio 1) shows a higher frequency of males siring offspring which is an indication of higher degree of male-male competition and scramble for access to females and fertilizing eggs. Figure 1b (sex ratio 3), on the other hand, shows mostly two peaks of two dominant males over the entire period.

Kinship and reproductive success

Females may choose mates on the basis of material benefits such as the quality of the male's territory or alternatively, males may be chosen because they have genes which will confer greater fitness on the female's offspring (Tregenza and Wedell, 2000). Many animals are known to bias social behaviour according to differences in their kinship to conspecifics

(Fletcher and Michener, 1987). Although the abilities of animals to detect smaller differences in their kinship to potential mates have been largely untested, numerous species have been observed to avoid mating with their first order (parents, full sibs) or second order (half-sibs) relatives (Barnard and Fitzsimons, 1988). In the present study, the degree of relatedness, as measured by the kinship coefficient, did not have any significant role in the reproductive success of males. There is increasing evidence that social factors are also important in influencing mate choice decisions (Westneat et al., 2000). For the evolution of inbreeding avoidance behaviour, the costs of inbreeding must exceed the costs of avoiding inbreeding (Koenig et al., 1998). In addition, as many fish are broadcast spawners releasing their gametes in water, fertilization and reproductive success could be affected by physical characteristics and presence of other competitive males in the surrounding. These factors can all confound the relationship between kinship and reproductive success. A better understanding of this relationship will undoubtedly broaden our understanding of mating systems and accumulation of inbreeding in this species and merits further investigation.

Concluding remarks

The results of this experiment showed enhanced reproductive success of relatively outbred individuals compared to inbred individuals. For males, the decline in reproductive success was higher in sex ratio 1 compared to sex ratio 3 indicating that the effect of inbreeding was magnified under higher male-male competition. This is consistent with many pervious studies that observed that aggressive encounters and higher male-male competition magnified the magnitude of inbreeding depression in reproductive success (Potts et al., 1994; Ekund, 1996; Meagher et al., 2000). To our knowledge this study is the first attempt to determine the effect of inbreeding on reproductive success in semi-natural conditions. The result uncovers a selective pressure that favors outbred males and that counterbalances the increase of inbreeding in a population.

Acknowledgements

This study is part of the first author's PhD study, funded by NUFFIC (The Netherlands organization for international cooperation in higher education), INREF-Pond, Wageningen University (http://www.inref-pond.org) and the WorldFish Center. We acknowledge the staff of The WorldFish Centre, Abbassa, Egypt for providing the necessary help and facilities during the experiments.

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Chapter 5

Prediction of Cannibalism in juvenile *Oreochromis niloticus* based on predator to prey weight ratio; and effects of age and stocking density

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***Aquaculture* 255 (2006), 314-322**

Abstract

Factors influencing size-dependent cannibalism in Nile tilapia, *Oreochromis niloticus* were investigated in controlled conditions. First, individual-based trials were conducted to develop a linear regression model to predict the occurrence of cannibalism based on body measurements. Oral gape (G, mm), body depth (D, mm) and weight (W, g) of 140 fish were measured and we estimated maximum prey weight (W_{prey}) for a given predator weight ($W_{predator}$): $Log_{10}W_{prey} = 1.03Log_{10}W_{predator} – 1.13$. This model was verified by conducting 76 single pair trials. The revised model based on observed cannibalism is: $Log_{10}W_{prey} = Log_{10}W_{predator} – 1.17$. Several experiments involving a group of prey and predators were conducted to elucidate the relative importance of factors that influence cannibalism. Cannibalism was significantly affected by stocking density, age of fish and predator: prey weight ratio. Logit models of mortality data of both the individual-based and observations based on a group of predators and prey estimated R_{50} (the predator: prey weight ratio at which an encounter of a predator and prey resulted in a predation success of 50%) to be 14.6 and 14.8 respectively. The results of these experiments can be used to reduce fry losses due to cannibalism.

1. Introduction

Cannibalism is the act of killing and consuming the whole or major part, of an individual belonging to the same species. Cannibalism can occur between unrelated animals, between siblings and can be exerted by parents on their offspring or vice versa (Baras and Jobling, 2002). It is a frequent phenomenon in fish especially in culture environments where fish are unable to escape predation via habitat segregation or mitigation.

A number of factors potentially influence the extent and rate of cannibalism in fish. These factors generally fall into two major categories, i.e. size-related and behavioural factors. Within the size-related category the principal cause of cannibalism is size variation within a cohort caused by genotypic differences which dictate differences in egg size (and hence larval size) and individual growth rates (DeAngelis et al., 1979). Factors which have been found to affect the behaviour of larvae and thus cannibalism are food availability, population density, refuge, water clarity, light intensity, feeding frequency and frequency at which alternative prey is presented (DeAngelis et al., 1979; Fox, 1975; Hecht and Appelbaum, 1988; Katavic et al., 1989).

Predator to prey size ratios vary substantially between different species and life stages; predominantly because cannibalism is governed by gape size limitations and allometric growth of mouth parts (Baras and Jobling, 2002; Lundvall et al., 1998; Qin and Fast, 1996). The size range of prey that a predator potentially can feed on is constrained by the predator's ability to capture and handle prey (Christensen, 1996). The lower prey size limit can be set by difficulties in retaining the prey (Persson, 1987) and by the predator's ability to detect the prey (Breck and Gitter, 1983). The upper limit is set by physical constraints of the predator, such as the size of the feeding apparatus (e.g. oral gape) (Hart and Hamrin, 1988; Hambright, 1991) and swimming capacity (Christensen, 1996). In Nile tilapia, low fecundity and the asynchronous reproductive cycles lead to episodic fry production (Jalabert and Zohar, 1982; Coward and Bromage, 2000) which will give rise to different hatching times and hence a wide size range. This size variation together with the aggressive nature of *O. niloticus* (Melard and Philippart, 1980) implies a potential for size-dependent cannibalism. Pantastico et al. (1988) reported that intracohort cannibalism was found in *O. niloticus* groups in which large size differences become established within age cohorts. This presents a problem in aquaculture. Macintosh and De Silva (1984) indicated that size-dependent cannibalism may account for an average loss of 10-35% of fry stock during the first 50 days of rearing. However, no information is available regarding the critical threshold in size difference above which cannibalism is likely to occur.

In this study, we present a simple regression model that predicts maximum prey weight for a certain weight of a predator based on body morphometric measurements; the model was evaluated with paired fish trials. Since these paired fish trials do not reflect the conditions in culture environment, we conducted several experiments to elucidate if the results of the model are still valid in situations where several predators and prey are involved. We focused on how varying sizes of fish (both predator and prey), different stocking densities, age of fish and tank volume affect predator-prey interactions and consequently the rate of cannibalism. Such information may help to understand cannibalism and will have practical application in mitigation of cannibalism in aquaculture.

2. Materials and Methods

2.1. General procedures

In all experiments, cannibalism trials were conducted by combining larger sized fish (hereafter *predator*) with smaller fish (hereafter *prey*) in the same aquarium. In the single pair trials, only one predator and one prey were involved. In the cannibalism trials involving a group of predators and prey, weight range within predator and prey groups in each trial were kept to a minimum by sampling near identical weights of both predators and prey (CV < 6.3%). All trials lasted for 24 hours. Fish were fed a commercial pellet (Feed type Nutra ABOOO, Feed size 0.4-0.7) at a daily ration 20g/kg$^{0.8}$ supplied by hand 4 to 5 times during the experimental period. Observation on agonistic and cannibalistic behaviour of both predators and prey was carried out immediately after combining predator(s) and prey(s) and during the first 10 minutes of the first four hours of the experiment. In each trial, prey percentage mortality was estimated as a fraction of the number of prey that had been cannibalized after 24 hours relative to the total number of prey stocked initially.

2.2. Experimental set up and data analysis

2.2.1. Cannibalism involving single predator and prey (Single pair trials)

2.2.1.1. Predator-prey morphometric model development

A predictive model for maximum prey size was developed based on measurements from 140 *O. niloticus* fry ranging from 14 to 100 mm total body length. All individuals were measured for total body weight (W, nearest 0.1 g), total body length (L), oral gape (G) and body depth (D) (nearest mm). L was measured as the distance from the tip of the snout to the end of the caudal fin; G (oral gape) as the maximum dorso-ventral dimension of the mouth which was measured by opening the mouth to its maximum vertical open-mouth position and D as the maximum body depth measured dorso-ventrally just anterior to the dorsal fin. It was assumed that a predator could swallow a prey with a body depth smaller than or equal to its maximum oral gape. Linear relations were developed between log-transformed values of $G_{predator}$ vs. $W_{predator}$ and D_{prey} vs. W_{prey}. The two equations were combined to create a theoretical equation relating W_{prey} and $W_{predator.}$ The resulting equation was used to predict the maximum prey weight (W_{prey}) for a given size of a predator ($W_{predator}$).

2.2.1.2. Verification of the morphometric model with paired fish

In order to verify the predictions given by the equation for maximum prey weight (W_{prey}), 76 single pair trials involving 23 sizes of predator (size range: 0.53 – 15.08 g) and 76 sizes of prey (size range: 0.03 – 1.20 g) were carried out in 20L aquaria at a temperature of 26-28° C and photoperiod of 12D: 12L. In each trial, a prey of known length and weight was paired with a predator of known length and weight. The fish were checked at regular intervals. If a prey had been eaten, its size was considered to be within the limits of predation for that particular sized predator and the predator was then given a somewhat larger individual prey. If the prey had not been eaten within the next two days, the prey was considered too large for that particular predator. In case of predation, an interval of 24 hours was maintained between consecutive trials to provide enough time for the predator to digest the consumed prey and to make sure that the predator was not satiated because of the preceding prey. The maximum prey size for a given predator weight was assumed to be between the largest consumed prey and the prey that was too large for that predator. The maximum prey weight was then calculated as the average between these two values. Mortality was scored on a binary scale (1= killed, 0= not killed). Generalized Linear Model (GENMOD) procedure incorporating a binomial model distribution with logit-link function (SAS, 1989) was used to analyze the relationship between mortality scores (response variable) and the predator: prey weight ratio (explanatory variable). This procedure models a linear relationship between the probability of mortality of a prey (in logit scale) and the predator to prey weight ratio. The fitted values of the logit model were transformed back into proportions to show the fitted curves in their sigmoidal form. The predator: prey weight ratio that resulted in probability of 50% mortality (hereafter R_{50}) of a prey was estimated. The model fitted was

$Y_i = \beta_0 + \beta_1 LogX_i + \varepsilon_i$ .... (Model 1)

Where, Y_i = the logit of probability of a prey being killed, β_0 = the intercept; β_1 = the regression coefficient of Y_i on $LogX_i$; X_i = is the predator to prey weight and ε_i = the residual error term.

2.2.2. Cannibalism involving group of predators and prey

In order to assess the validity of the results of our regression model for practical fish culture conditions, several experimental trials involving groups of predators and prey were conducted. In these trials we investigated the effect of different predator to prey weight ratio, stocking density, age of fish and tank volume on the rate of cannibalism.

2.2.2.1. Effect of predator to prey weight ratio on cannibalism

Predator: prey ratio could be used to predict the success or failure of a predator in individual-based predation models. To assess if this ratio also holds true in culture conditions in which several predators and prey are involved, a total of 63 trials involving different sizes of predators and prey were conducted. In each trial fifteen predators and fifteen preys were stocked in 30L glass aquaria. Various combinations of predator and prey weights were used to generate categories of predator: prey ratios ranging from 3 to 45. The average weights of predators and prey ranged from 0.7 to 24 g and 0.09 to 0.77 g respectively. Predator and prey originated from unrelated families or in some cases they were half sibs. GLM procedure was used to test the effect of predator: prey weight ratio and family relationship on the rate of cannibalism. The model fitted was

$Y_{ij} = \mu + F_i + \beta LogX_j + \varepsilon_i$... (Model 2)

Where Y_{ij} = proportion of prey killed; μ = overall mean; F_i = family relationship between predator and prey (i = half sibs, unrelated), X_j = a co-variable of the predator to prey weight ratio; β = regression coefficient of Y_{ij} on $LogX_j$ and ε_{ij}= the residual error term.

Furthermore, Generalized Linear Model (GENMOD) procedure with logit-link function (SAS, 1989) was used to analyze the relationship between mortality rates and the log-transformed predator: prey weight ratio. The same procedure was followed as in Model 1 for fitting a logistic regression and R_{50} was also estimated. The following model was eventually fitted:

$$Y_i = \beta_0 + \beta_1 LogX_i + \varepsilon_i \ldots\ldots (Model\ 3)$$

Where Y_i = the logit of probability of a prey being killed; β_0 = the intercept; X_i = predator to prey weight ratio; β_1 = the regression coefficient Y_i on $LogX_i$; and ε_i = the residual error term.

2.2.2.2. Effect of age and density on cannibalism

These trials were designed to investigate the effect of age on the rate of cannibalism in larvae over the course of their early life stages. Two batches of older fish (predators) and one batch of younger fish (prey) with age difference of two weeks were produced for this experiment. When the prey were 7 days and the predator 14 days post hatching, the first set of cannibalism trials were conducted (Predator: average weight = 0.79 g, SD = 0.073; prey: average weight = 0.058 g, SD = 0.0098). Different fish from the same group of predators and prey were used to conduct three set of trials on three consecutive weeks. The average weight ± SD of the predators and prey for the trials were as follows: trial II predator (1.69 ± 0.08) and prey (0.14 ± 0.02); trial III predator (3.98 ± 0.22) and prey (0.36 ± 0.03) and trial IV predator (3.06 ± 0.1) and prey (0.4 ± 0.11). In total 24 trials were conducted in which fish were stocked at three different densities of 0.33, 1 and 2 fish/L in 30L aquaria. GLM procedure of SAS (1989) was used to test the effect of age, density and predator to prey weight ratio on the rate of cannibalism by using the following model:

$$Y_{ijk} = \mu + A_i + D_j + \beta LogX_k + \varepsilon_{ijk} \ldots\ldots (Model\ 4)$$

Where Y_{ijk} = Proportion of prey killed; μ = overall mean; A_i = age group of predator and prey; D_j = density of fish (j = 0.33, 1, 2 fish/L); X_k = a co-variable of the predator to prey weight ratio; β = regression coefficient of Y_{ijk} on $LogX_k$; and ε_{ijk} = the residual error term. Scheffe's multiple-range test was also used for multiple comparisons of means.

2.2.2.3. Effect of stocking density and tank volume on the rate of cannibalism

The effect of stocking density and tank volume on the rate of cannibalism was evaluated in aquaria with different volumes (20, 30, 100 and 300L) and stocking densities of 0.33, 1 and 2 fish/L. In total 49 trials with predator: Prey ratios ranging from 1:5 to 1:40 were conducted (See Table 1 for details). GLM procedure of SAS (1989) was used to test the effect of volume, density and predator to prey weight ratio on the rate of cannibalism. The model fitted was

$$Y_{ijk} = \mu + V_i + D_j + \beta Log X_k + \varepsilon_{ijk} \text{ ....(Model 5)}$$

Where Y_{ijk} = Proportion of prey killed, μ = overall mean, V_i = volume of tank in liters (i = 20, 30, 100, 300) D_j = density of fish (j = 0.33, 1, 2 fish/L), X_k = a co-variable of the predator to prey weight ratio, β = regression coefficient of Y_{ijk} on $LogX_k$, and ε_{ijk} = the residual error term.

Table 1. Summary of the experimental design on effects of density and tank volume on the rate of cannibalism

Volume	No. of fish		Number of trials	Density (No. of fish/L)	Predator: prey weight ratio range
	Prey	Predator			
20	10	10	13	1	5 - 25
30	5	5	10	0.33	5 - 21
	15	15	10	1	7 - 12
	30	30	8	2	8 - 13
100	50	50	4	1	3, 37, 40
300	50	50	2	0.33	21, 22
	150	150	2	1	34, 35

3. Results

3.1. Cannibalistic behaviour

In both the individual-based trials and in trials involving group of predators and prey, fish showed similar cannibalistic and agonistic behaviour. After combining, predator and prey occupied different corners in the aquaria. The prey tended to aggregate near the water surface while the predators remained on the opposite lower corner of the aquarium. After a few minutes, the predators started chasing the prey. The predators attacked the prey from different orientations (front, tail or lateral) consuming the prey head-first or tail first. Predator attacks on prey were either ‘one to one’ where one predator was attacking one prey or ‘group cannibalism’ where several fish were attacking one victim. In general, two types of cannibalism were observed: either prey were caught by the tail or they were swallowed whole. The latter type of cannibalism occurred when the predator to prey ratio was very high. In most cases attacks occurred within the first ten minutes and the rate of attack subsided with time.

3.2. Predator-prey morphometric model for single pair trials

The linear regression equations of *O. niloticus* body depth (D) and oral gape (G) on body weight were

$Log_{10}G_{predator} = 0.37Log_{10}W_{predator} + 0.65$ ($r^2 = 0.96$)……………………………………….(1)

$Log_{10}D_{prey} = 0.36Log_{10}W_{prey} + 1.06$ ($r^2 = 0.98$)……………………………………………...(2)

Because oral gape of a predator largely determines maximum prey size, we assumed that a predator could swallow a fish with a body depth smaller than or equal to its maximum oral gape. Combining 1 and 2 results in an equation relating W_{prey} and $W_{predator}$:

$Log_{10}W_{prey} = 1.03Log_{10}W_{predator} – 1.13$……………………………………………………(3)

3.3. Model verification with paired fish

Figure 1 shows the relationship between predator weight and prey weight in *O. niloticus* fry and fingerlings. Observed predation by larger *O. niloticus* on smaller ones in paired fish trials showed that there was a slight over estimation of prey size predicted by the morphometric model. For example, a predator of about 15 g was predicted to consume a prey of about 1.21g, but it could actually consume a prey of only 0.95 g. The equation used to predict maximum prey weight for a given size predator was, therefore, revised as: $Log_{10}W_{prey} = Log_{10}W_{predator} – 1.17$**.** Comparison of the regression coefficient and intercept of both observed and predicted cannibalism showed that there was no significant difference between both lines ($P > 0.01$). R_{50} for the individual-based trials as estimated from the regression line (Figure 1) and from the logit model (Figure 2) were 14.8 and 14.6 respectively.

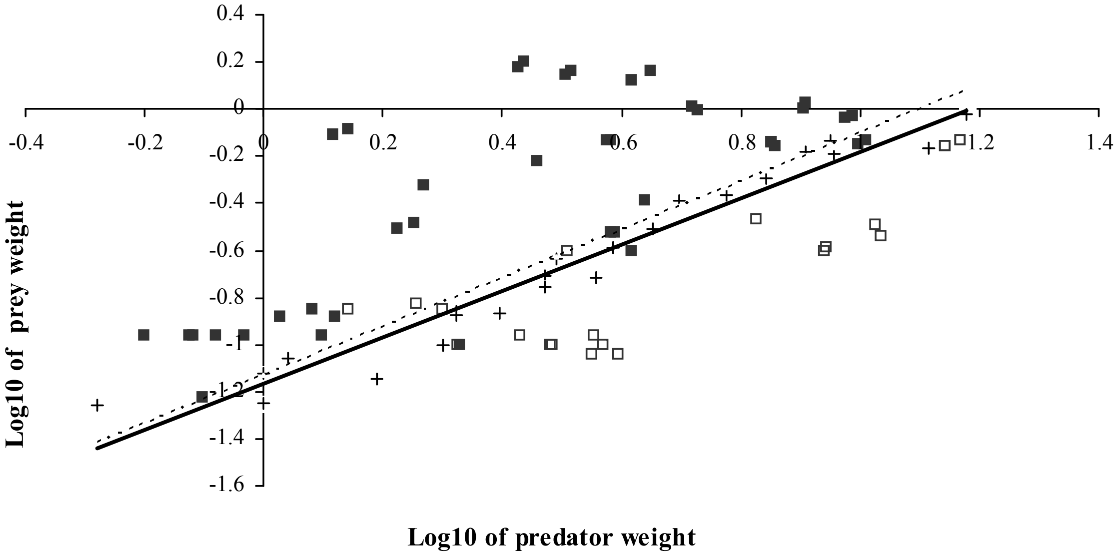

Figure 1. Relationship between predator and prey weights in *Oreochromis niloticus*. The cross symbols (+) represents the upper limit of prey weights which were consumed by a given weight of a predator as determined from pairwise predation trials. The upper size limit of prey was calculated as the average of the largest prey consumed and the prey that was too large to be consumed. The solid line is regression of observed maximum prey weight on predator weight ($R^2 = 0.95$). The broken line represents the regression of predator weight on prey weight as predicted from the morphometric model (equation 3). The black squares (■) and open squares (□) symbols respectively represent 0% and 100% mortalities in the experiments involving groups of predators and prey.

3.4. Effect of predator to prey size ratio on the rate of cannibalism in groups of fish

ANOVA analysis (Model 2) revealed a significant effect of predator: prey weight ratio ($P < 0.001$) on the rate of cannibalism. There was no significant effect of family relationship on the rate of cannibalism ($P = 0.519$) (Table 2). That is cannibalism was not influenced by whether predator and prey were half sibs or coming from unrelated families.

The relationship between predator to prey weight ratio and rate of cannibalism in groups of Nile tilapia is depicted in Figure 2. A logit model was fitted to the data and the R_{50} was estimated to be 14.8 (with 95% confidence interval of 11.00 to 18.60). Model 3 attributed approximately 60% of the observed variance in mortality to the predator: prey weight ratio. Fifty four of the 63 observations could be explained by the predator to prey ratio threshold of 14.8; the remaining 9 trials produced results different from the predictions which could not be explained by the predator to prey weight ratio. For example, weight ratios of 6.5, 10.4, 11.5

and 14.3 resulted in average mortalities of 60, 73.3, 60 and 100% respectively. Two trials with weight ratios of 16.5 and 21.4 resulted in zero mortality.

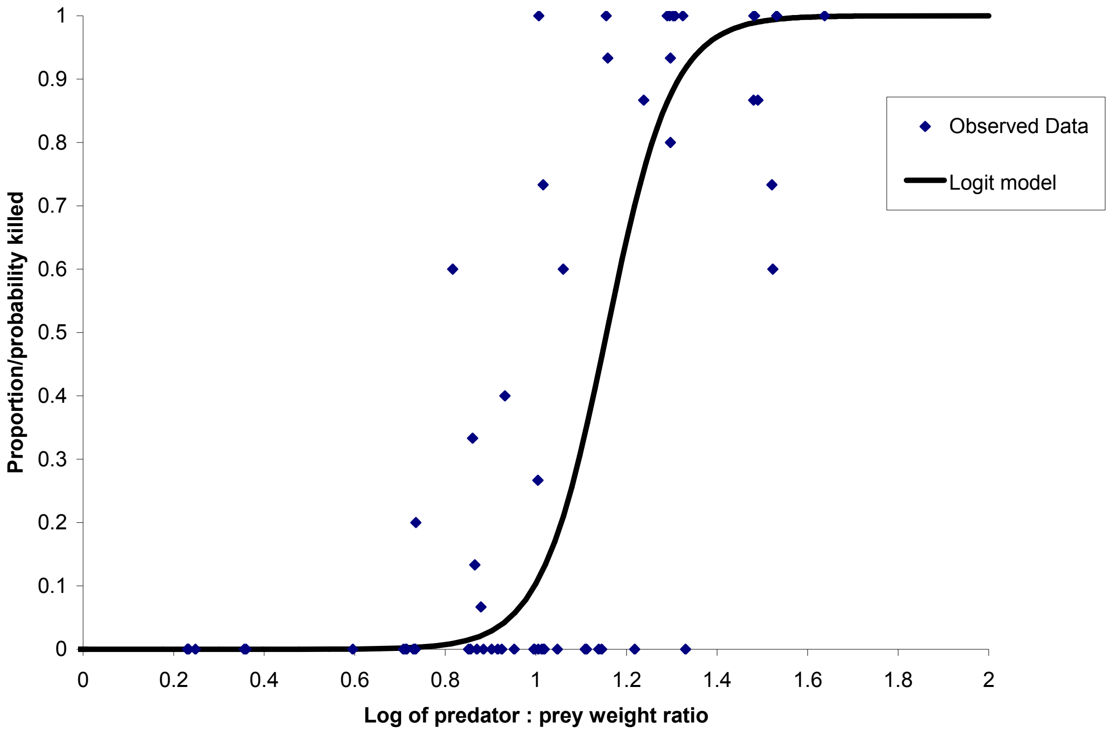

Figure 2. Predicted probabilities and observed proportions (dots) of cannibalisms in groups of 15 prey and 15 predators of *O. niloticus*. The sigmoid curve was fitted to the observed data by transforming the logit probability of mortality in to proportions.

Table 2. Marginal (Type III) mean squares and *P* values of the effects in Model 2 on the rate of cannibalism in Nile Tilapia.

Source	DF	Mean square	*P*
Family relationship	1	0.0432	0.519
Log(Predator: prey weight ratio)	1	5.398	< 0.001

3.5. Effect of age and density on the rate of cannibalism

Analysis of ANOVA (Model 4) revealed significant effects of predator to prey weight ratio (*P* = 0.003), density of fish (*P* = 0.0005) and age group (*P* = 0.0006) on the rate of cannibalism (Table 3). Model 4 explained approximately 77% of the observed variance of mortality.

Parameter estimates of age group on the response variable (mortality proportion) ranged from -0.30 to -0.66. Multiple comparisons of means within the same age group showed that cannibalism in trials with density of 2 fish/L was significantly (*P* < 0.01) higher than mortalities in experiments with prey densities of 0.33 and 1 fish/L. There was no significant difference between prey densities of 0.33 and 1. Parameter estimates for density 0.33 and 1 fish/L respectively were -0.49 and -0.18 respectively.

Table 3. Marginal (Type III) mean squares and *P* values of the effects in Model 4 on the rate of cannibalism in Nile Tilapia.

Source	DF	Mean square	*P*
Age group	3	3.313	0.0006
Density (No. Fish/Liter)	2	4.165	0.0005
Log(Predator: prey weight ratio)	1	4.104	0.003

3.6. Effect of stocking density and tank volume on the rate of cannibalism

The ANOVA result of the experiment examining the effect of stocking density and tank volume is shown in Table 4. There were significant effects of stocking density (*P* = 0.001) and predator to prey weight ratio (*P* < 0.0001) on the rate of cannibalism. Volume of culture tank had no significant effect on cannibalism at P < 0.05 (*P* = 0.08). Model 5 explained about 80% of observed variance of mortality. The mortality parameter estimates for the tested volumes ranged from -0.03 to -0.23. The mortality proportion estimates for density compared to 2 fish/L were -0.38 and -0.21 respectively. The proportion of mortality for densities 0.33, 1 fish/L was significantly lower than for 2 fish/L.

Table 4. Marginal (Type III) mean squares and *P* values of the effects in Model 5 on the rate of cannibalism in Nile Tilapia.

Source	DF	Mean square	*P*
Volume	3	1.586	0.080
Density (No. Fish/Liter)	2	5.137	0.001
Log(Predator: prey weight ratio)	1	59.740	< 0.0001

4. Discussion

With most predator-prey relationships, morphological characteristics of predator feeding apparatus are important constraints determining maximum prey size (Dabrowski and Bardega, 1984). Piscivorous fish are gape-limited predators. Because prey are generally swallowed head- or tail first, their body depth (measured dorso-ventrally) relative to the size of a piscivore's mouth determines whether they can be ingested (Hambright, 1991). Thus, in any particular habitat prey with body depths greater than the largest piscivore gape are invulnerable to ingestion. The results from this experiment are in agreement with the general fact that there is high correlation between dimensions of body parts. Size of prey consumed by predators could be predicted from that predator’s oral gape. Morphometric prediction of cannibalism is species-specific because different species have different mouth morphology. However, the model approach can prove useful for predicting cannibalism for larvae of known size distribution.

Body size is known to play a crucial role in predator-prey interactions. Size relationship between predator and prey is perhaps the single most important component in capture success (Lundvall et al., 1998). Predator: prey size ratio is an important variable affecting cannibalism (Qin and Fast, 1996) and it is generally assumed that cannibals can capture only victims that are smaller than some critical body size owing to morphological limitations of the predator and escape response of the prey (Christensen, 1996). The upper limit to victim size is often assumed to be a fixed ratio of cannibal size (DeAngelis et al., 1979) but the precise relationship between cannibal size and victim size is rarely known. In this study, predator to prey ratio has been pointed out as the most important factor affecting rate of cannibalism in *O. niloticus.* In the individual-based trials, an encounter of prey and predator with a weight difference of 14.6 resulted in a 50% probability of the prey being killed by the predator ($R_{50} = 14.6$). Individual-based modeling approaches have often assumed threshold values of prey size/predator size ratios to determine capture of juvenile fishes given an encounter with a piscivore, with prey size/predator size ratios below threshold values resulting in 100% capture efficiency and those above always resulting in failed capture attempts (Madenjian et al, 1991). For the cannibalism observations involving several fish, a critical predator to prey weight ratio of 14.8 was calculated as a critical ratio at which 50% of predation attempts by the predator resulted in successful predation of the prey. Although predator-prey interactions involving several fish are often complex, the results of the individual-based approach and the trials that involved multiple prey and predators had nearly identical R_{50} (14.6 and 14.8). Previous work has demonstrated the potential effectiveness of size-dependent information obtained from laboratory predation experiments in predicting the outcome of population-level interactions (Rice et al., 1993). However, the use of discrete values of relative fish size (predator to prey size ratio) to determine capture success should be carefully handled as it ignores the dynamic nature of size-dependent predation process. This is particularly important, given that the output (cannibalism) could be extremely sensitive to the variation in this parameter (predator: prey ratio) (Scharf et al., 1998). The difference in predator attributes such as swimming speed and search pattern may result in differential encounter rates with larval prey fish, leading to considerable effect on capture success and prey survival (Cowan et al., 1996).

In fishes, early ontogenic changes in both size and morphology have major implications for predator avoidance capacity (Fuiman and Magurran, 1994). Fish larvae possess several sensory systems that are presumed to be important in predator detection and avoidance (Blaxter, 1986). An increase in absolute body size also acts on predator-prey interactions, as larger locomotor muscles provide more power to escape predators, and size also limits the numbers of potential gape-limited predators. Together with the morphological and developmental consequences of growth, the behavioural repertoire of a fry also increases through learning (Lundvall et al., 1998). Prey larvae have improved ability to avoid predation as they grow bigger and swim faster (Li and Mathias, 1982). Our results are in agreement with the fact that prey fish become less vulnerable as they grow older. Prey fish have either developed a mechanism of predation escape as they grow older or the increase in body size of prey has led to the decrease in the predator to prey ratio and hence resulted in lesser cannibalism. However, at higher predator to prey weight ratio cannibalism was still considerably high despite the age of the fish.

In all species in which cannibalism has been investigated a positive density dependent correlation appears to exist (Hecht and Pienaar, 1993). In many cases in which cannibalism appears to be affected by fish density, a confounding factor is food availability. The influence of stocking density on cannibalism at larval stages had already been evidenced in a number of studies (Li and Mathias, 1982; Giles et al., 1986; Hecht and Appelbaum, 1988; Smith and Reay, 1991). Macintosh and De Silva (1984) reported that under conditions of moderate crowding and low food input cannibalism may become a significant cause of mortality in tilapia fry. Our results also demonstrated that prey vulnerability was increasing with increasing density of fish and the effect of density was significant on the rate of cannibalism. Higher density of either cannibal or prey promotes cannibalism through increased encounter rate between predators and prey (Smith and Reay, 1991). In addition to capture success, differences in encounter rate can also determine the outcome of predator-prey interactions. Differential abundance or availability of prey can lead to higher encounter rates and increased predation (Juanes, 2002).

In various taxonomic groups, socially organized animals frequently show recognition of close relatives. A degree of kin recognition may operate in at least some fish species, with the result that cannibalism of close relatives is avoided or suppressed (Smith and Reay, 1991). However, no significant difference was noticed in cannibalism between related (half sib) predators and prey and those coming from unrelated families. This was in agreement with findings of McKaye and Barlow (1976) which demonstrated that kin recognition minimizes filial cannibalism in Cichlids. Although we can not strictly conclude that tilapia larvae were unable to discriminate between half-sibs and non-related fish; we can cautiously state that related predators and prey (halfsib) are probably not less cannibalistic when compared to unrelated predators and prey. Further investigation should be carried out to elucidate the effect of family relationship on the rate of cannibalism in Nile tilapia.

In both the individual-based trials and trials that involved groups of predator and prey, the highest intensity of agonistic behaviour and cannibalism occurred in the first hour of the experiment. Although agonistic behaviour became less intense, it did not disappear during subsequent hours. It is not clear whether decreasing trend of cannibalism over the experimental period is due to familiarization, establishment of hierarchical stability or satiation of the predators. Our previous results (unpublished data) indicate that familiarization of predators and prey did not have any mitigating effect on confrontation and cannibalism. These results do not support the findings of Giaquinto and Volpato (1997) which showed that in juvenile Nile tilapia chemical communication decreases aggression among conspecifics by modulating conspecific recognition, thereby increasing hierarchical stability and decreasing confrontation.

This study showed that cannibalism among larvae might be triggered by many husbandry-related factors but that size variation has an overriding effect on the rate of cannibalism. In other words, cannibalism can not be avoided if substantial size heterogeneity exists among fish. DeAngelis et al. (1979) showed that initial size variation was more important than alternative food availability in controlling cannibalism. An optimal fry loss reducing strategy would be to maintain fry at conditions which discourage size heterogeneity in combination with size grading. The predator to prey weight ratio of 14.6 can be considered as critical threshold for screening size heterogeneity and predicting the level of fry losses due to cannibalism.

Acknowledgements

This study is part of the first author's PhD study, funded by NUFFIC (The Netherlands organization for international cooperation in higher education) and INREF-Pond of Wageningen University (http://www.inref-pond.org). We would like to acknowledge Menno ter Veld, Sietze Leenstra for their technical assistance.

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Chapter 6

General discussion

Introduction

Mating systems in fish have received little attention despite the significant impact they can have on population dynamics and conservation biology (Rowe and Hutchings, 2003) of both captive and wild populations. Broodstock management programmes aiming at either genetic improvement (for commercial aquaculture) or genetic homeostasis (for restocking and/or maintenance of an endangered species) have a breeding plan comprised of selection of parents (broodstock) for the next generation and systems for mating the selected parents (Turner and Young, 1969). Breeding schemes employing mass spawning have two constraints: 1) Mate selection and mating of the animals is not controlled and hence a small number of breeding individuals may account for a large proportion of the subsequent generation; and 2) high fecundity in most fish allows the use of small number of parents and high selection intensities. These two factors are generally recognized as the main sources of inbreeding in hatchery-produced fish populations (Bentsen and Gjerde, 1994; Gjerde et al., 1996; Gjedrem, 1998). High rates of inbreeding can affect survival, reproduction, growth, the expression of genetic defects and the level of genetic variability (Kincaid, 1983).

In *Oreochromis niloticus,* fry production is typically achieved by mass-spawning of males and females stocked in large hapas, earthen ponds or tanks. Reproductive competition is expected in a mass spawning system due to asynchronous spawning of females and the aggressive nature of males. However, the effect of such reproductive competition on parental contribution and other population parameters has not been studied previously. One of the objectives of this thesis was to increase our understanding of the natural mating system in *O. niloticus,* which is a primary determinant of reproductive success. In chapters 2 to 4, reproductive variance and other population parameters (individual variation in reproductive success, effective population size and the rate of inbreeding per generation) were quantified and the effects of inbreeding on morphological and fitness traits were investigated. In chapter 5, the consequences of episodic fry production on size heterogeneity and cannibalism were explored. In the present chapter, the results described in previous chapters will be discussed and implications of the results will be explored in view of refining breeding schemes based on mass spawning.

Mating systems

A wide range of genetic mating systems have been documented in nature and are believed to influence many important genetic and evolutionary attributes: genetic diversity (Jarne and

Städler, 1995); inbreeding, hybridization (Stephens and Sutherland, 2000) and intensity of sexual selection (Neff, 2001). A thorough understanding of mating systems is, therefore, important for many aspects of management of both domesticated and natural populations.

The evolution of mating systems in cichlid fishes has proceeded from monogamy with biparental care to polygamy with maternal or paternal care (Barlow, 1964; Gittleman, 1981). Polygamous mouth brooding cichlids have been studied closely in nature. Males are usually larger and more colorful than females. Polygynandry prevails in that males mate with several females, and females may deposit their eggs among more than one male when males are close together (Barlow, 1991). Males provide no resource and the rewards of promiscuity for males are undisputed because their reproductive success is directly related to the number of females/eggs inseminated. Females, on the other hand, have high parental investment and are expected to be choosy. The adaptive significance of females mating with multiple males is, therefore, less clear (Parker, 1992). Several hypotheses have been formulated claiming that promiscuous females secure substantial advantages. Benefits may be direct, such as fertility insurance (Birkhead and Fletcher, 1995) or genetic benefits such as compensation for low quality males (Kempenaers et al., 1992), avoidance of genetic incompatibility (Zeh and Zeh, 1996) and increased heterozygosity (Brown, 1997). It has also been proposed that female promiscuity reduces fitness costs of inbreeding by increasing the probability of producing outbred offspring (Stockley et al., 1993). In chapter 2 and 4 of this thesis, it was shown that *O. niloticus* utilize several different mating systems ranging from single pair mating (monogamous) to polygamous matings. Results in chapter 4 also revealed that sex ratio and number of competitors influenced the prevalence of the different mating systems in tilapia. The promiscuity of males and the stronger male-male competition in the presence of higher density of males is consistent with many other findings and it was not surprising as it is a mechanism to increase their reproductive success. A question that arises from our data in this thesis is why females mate with multiple males? It has been hypothesized that females may mate indiscriminately to bet-hedge against male infertility and to enhance genetic quality of offspring. In this thesis, we are not able to discern the advantages of multiple mating for females. For example in Chapter 2, there was no significant difference in hatching percentages between multiply sired spawnings (76.9 ± 13.4) and spawnings sired by a single male (75.5 ± 13.0) (results not presented) suggesting that females did not have fertilization advantages by mating with multiple males. Given the fact that only few females spawned at a time while practically all males were sexually active, we would think that females should be choosy. However, with the crowded environment and intense competition between males in the breeding hapas, we doubt if females might be able to exercise choosiness and we cautiously say that multiple matings were probably more male-driven.

Reproductive success and effective population size

In an idealized population mating is random i.e. reproductively active individuals have equal opportunity to mate and contribute equally to the gene pool. However, theory predicts that differences between the sexes in potential reproductive rate and a sex ratio biased strongly towards males should result in intense male competition, polygynous mating and high variance in male reproductive success (Kvarnemo and Ahnesjo, 1996). The mass spawning experiments (chapter 2 and 4), have either female-biased sex ratio (sex ratio 2 and 3: more females than males) or unbiased (sex ratio 1: equal number of females and males). However, the operational sex ratio (the ratio of sexually active males to fertilisable females) in all the sex ratios was biased towards males as only few females spawn at a time while most males are sexually active. Such male-biased sex ratios caused an intense competition between males with stronger male-male competition in the sex ratio 1 compared to sex ratio 3 (Chapter 4: Figures 1 and 2) as more males are present in sex ratio1. Moreover, better conditioned/less inbred males acquire a higher proportion of offspring. This could be either due to their direct advantage in male-male competition leading to higher access to females or indirectly due to higher sperm volume and quality which in turn confer an advantage in sperm competition and hence acquisition of fertilizations. Overall, the reproductive success of males was highly skewed with around one third of the males contributing 70-93% of the offspring analyzed. In comparison female contribution was much more balanced with most females (70-92%) spawning at least once during the spawning period. As shown in Table 1, male reproductive variance (σ_{km}^2) was by far greater than female reproductive variance (σ_{kf}^2) indicating that male contribution was more skewed than that of females. There was a high discrepancy between the census population size (N) and the effective population size, N_e (Table 1). The factors causing a reduced magnitude of N_e were the high variances in male contribution (σ_{km}^2) together with the small number of contributing males. Matocq (2004) reported that a decrease in N_e can be caused by a number of behavioural and demographic factors operating within populations. Specifically, strong skew in male reproductive success, as would be expected in polygynous mating systems, could cause a substantial decrease in N_e relative to the census population size. A N_e/N ratio of 0.30 - 0.44 in this study indicates that less than 50% of the parents are contributing to the next generation and this suggests that the population is experiencing higher rates of genetic drift, being highest in the population with a female to male sex ratio of 3 (Table 1). This low N_e equated to a rate of inbreeding of 3.0 - 4.4% per generation, which is 2 -2.5 times the rate of inbreeding expected in an idealized population ($\Delta F = 1/8N_m + 1/8N_f$: Falconer, 1989).

Table 1. Census number, reproductive variance, effective population size and rate of inbreeding for the spawnings conducted in 2003 and 2005.

Year	N[1]	Sex ratio	σ^2_{km}	σ^2_{kf}	N_{em}	N_{ef}	Total N_e		ΔF		N_e/N
		(F to M)					Predicted[2]	Idealized[3]	predicted[4]	idealized[5]	
2003	37	2:1	2094	149	5.0	17.8	15.6	32	0.0309	0.0156	0.43
2003	37	2:1	725	86	5.4	15.8	16.1	32	0.0301	0.0156	0.45
2005	36	3:1	10165	380	3.2	17.1	10.8	27	0.0437	0.0185	0.30
2005	36	1:1	1309	460	5.5	10.2	14.3	36	0.0341	0.0139	0.40

N_{em} and N_{ef} are effective number of males and females respectively; σ^2_{km} and σ^2_{kf} are reproductive variances for males and females respectively

[1] *Census size is the total number of males and females*

[2, 3] *Calculated using the formula $N_e = 4N_{em}N_{ef}/(N_{em} + N_{ef})$. For predicted total N_e, N_{em} and N_{ef} are used; for idealized total N_e, the census number of males and females was used*

[4, 5] *ΔF was calculated using $\Delta F = \frac{1}{2}\sum_{Parents} c_i^2 - \frac{1}{4}(\bar{c}_m)^2 - \frac{1}{4}(\bar{c}_f)^2$, for predicted ΔF contribution of parents was obtained from parentage analysis; for idealized ΔF equal contribution of parents was assumed (for details of this formula see Chapter 2)*

Harmful effects of inbreeding have been noticed in numerous animal and plant species. Results in chapter 3 and 4 of this thesis show that inbreeding significantly reduces the phenotypic performance of a number of traits important in tilapia aquaculture (See Table 2). Taking the ΔF estimate of sex ratio 3, the decline in phenotypic performance of different traits can be extrapolated for breeding schemes that employ natural mating at that sex ratio. An inbreeding rate of 4.4% per generation can cause a decline in fry survival of 2.4% (or ≈ 4% of mean survival); a decline of the number of hatched fry by 42 (11% of the mean); and a reduction in fecundity by 33 eggs per female (5% of the mean). Such levels of inbreeding can also lead to depression of male reproductive success by up to 18%, the depression being much higher under more intense male-male competition (sex ratio 1, for example). The results of this thesis have generally shown that inbreeding affects not only traits at early stages but also inbred animals that survive to adulthood may still suffer reduced fitness via poor performance in mating competition and reduced fecundity. Lower reproductive success of inbred adults under natural conditions suggests the presence of a selective pressure that favours outbred adults and uncovers a mechanism that counterbalances the accumulation of inbreeding in natural mating systems. In tilapia aquaculture that employs mass spawning for fry production, the accumulation of inbreeding and its actual impact on different traits could, therefore, be lower than predicted. However, under the precautionary principle, managing of ΔF should still be an essential component in breeding programmes because as demonstrated in this thesis increased inbreeding has detrimental consequences for survival, reproduction as well as production traits.

Table 2. Mean ± SD of trait, regression coefficient of inbreeding (F %) and genetic depression in phenotypic performance per 4.4% increase in the level of inbreeding.

Trait	Trait Mean ± SD	Regression coefficient (F%)	Genetic depression per 4.4% of F[a]
Fry survival (%)	63.6 ± 14.7[1]	-0.55	2.4
Initial weight (g)	2.1 ± 1.7[1]	-0.14	0.6
Tot. number of hatched fry	384 ± 277[1]	-9.6	42.2
Female fecundity	674 ± 423[2]	-7.4	32.6
Male RS* (proportion)	0.10 ± 0.24[2]	-0.04	0.18 (or 18%)

* RS =reproductive success [a] Decrease in phenotypic performance per 4.4% increase in inbreeding
[1] data from chapter 3; [2] data from chapter 4

Size heterogeneity and cannibalism

Size dependent competition and cannibalism is a common phenomenon in tilapia. Size heterogeneity in hatcheries is the result of asynchronous reproductive cycles and initial size advantages resulting from environmental, maternal and direct genetic effects and competition or social interactions. Hatcheries combine fry of different families hatched at different times and this increases size dependent aggression and cannibalism. Results in Chapter 5 show that cannibalism among larvae might be triggered by many husbandry-related factors but that size variation has an overriding effect on the rate of cannibalism. The probability that a prey gets killed was low at a predator to prey weight ratio of less than ten. However, for a ratio of 10-15 there was a lot of variability in the outcome of an encounter between a prey and predator. A predator to prey ratio of 15 was considered as a critical ratio where an encounter of a predator and a prey 15 times smaller resulted in cannibalism-induced mortality of 50 % of the encounters. This has a practical implication as weight ratio of 15 is common in culture facilities with mixed and ungraded fish. Yousif (2002) conducted different experiments to evaluate the effects of density, water flow and feeding frequency on size heterogeneity and reported a coefficient of variation of up to 80% for weight and a biggest to smallest fish weight ratio of up to 16 times. Size heterogeneity has two major implications for fry rearing hatcheries. First it results in direct economic losses caused by cannibalism and fry losses. According to Macintosh and De Silva (1984) it could cause a fry loss of up to 35% in the first 50 days of rearing. Secondly, cannibalistic fish exhibit increased robustness and vigour, a reduction in developmental time, increased somatic growth rates, enhanced gonadal development (Li and Mathias, 1982; Meffe and Crump, 1987). Under such circumstances, fish breeders practising mass selection might inadvertently select for cannibalism as large size, faster growth rate and robustness are those phenotypic traits which a breeder desires in the selected broodstock. Many earlier mass selection schemes for growth in tilapias have shown lack of positive response to selection (Hulata et al., 1986; Teichert-Coddington and Smitherman, 1988; Huang and Liao, 1990). This lack of positive response has been attributed to depletion of genetic variation (Hulata et al., 1986); and to non-heritable maternal effects and size-dependent competition and cannibalism (Moav and Wohlfarth, 1984; Doyle and Talbot, 1986; Ruzzante, 1993). Effects of initial size advantages are more crucial when selection is practiced early at the fry stage resulting in selection of cannibalistic fry for grow-out. Initial size advantages might also persist and affect the weight at harvest. In *O. niloticus*, initial weight has been reported to significantly affect the survival and harvest weight (Charo-Karisa, 2006). Mass selection under such size heterogeneity might lead to selection of initial weight advantages which originate from cannibalism or rather than genetic merit. To minimize such non-genetic variations, a selection technique based on collimation (early size grading and culling of large fry) was proposed by Doyle and Talbot (1986). The effect of collimation is to increase the ratio of genotypic to phenotypic variance (i.e. heritability) and therefore increase the predicted response to directional selection. One generation of size-specific mass selection in combination with collimation procedure resulted in a significant response to selection of 8% for standard length and 29% for weight relative to the control (Basiao et al., 2005). Our results on the critical size ratio for cannibalism (Chapter 5) can be practically used for grading fry at early stages, thereby reducing direct fry losses due to cannibalism and improving the efficiency of mass selection schemes in tilapia by reducing the non-genetic variance.

Implications of natural mating systems for genetic diversity

Mating systems influence the genetic diversity within populations. Therefore, the genetic consequences of mating systems should be considered in the development of captive breeding plans and conservation of natural populations.

Implications for aquaculture stocks

Aquaculture is currently the fastest growing sector of food production in the world and by 2002 its contribution to the total fish and shellfish consumption was approximately 30% by weight (FAO, 2004). The majority of global aquaculture production (90%) is currently in developing countries (Hishamunda and Ridler, 2002). In 1993, less than 1% of the aquaculture production worldwide originated from genetically improved breed farm breeds (Gjedrem, 1997). Most aquaculture stocks currently used in developing countries are genetically similar to wild, undomesticated stocks. Anecdotal and empirical evidence even exists for substantial (up to 40%) declines in growth among *Oreochromis* captive populations in both large and small-scale fish farms in Africa (Brummett and Ponzoni, 2004; Brummett et al., 2004). Eknath (1991) reported the genetic deterioration taking place in hatcheries in India due to poor broodstock management. The experience of selective breeding in tilapias has been reviewed by Penman and McAndrew (2000) who concluded that the low heritability for growth observed in many trials were an outcome of the low genetic variation typical of the cultured stocks used. Although inappropriate husbandry practices might have also contributed to the overall decline in growth performance, most of the blame has been put on the genetic deterioration of stocks. Monitoring of genetic changes in populations undergoing selection have been done using microsatellite markers and results have revealed reduction in some genetic variability indices (number of alleles, H_e) and an increase in the degree of inbreeding by 107.9% after four generations of mass selection for growth in Nile tilapia (Romana-Eguia et al., 2005). Genetic diversity is the basis of the potential of species to respond to environmental changes and determines a species ability to adaptively cope with natural selection. It is also crucial in breeding programmes because it is critical for both the short term and long term limits of response (Falconer, 1989). To ensure the highest probability of success for breeding programmes, priority must be given to the maintenance of genetic diversity. In mass spawning, this can be achieved by different manipulations such increasing the effective population size or by maintaining separate lines of breeding stock and occasionally exchanging breeders between these separate lines.

Increasing effective population size (N_e)

Effective population size defines the effective number of individuals contributing to the next generation and helps to predict fixation probability of favorable and deleterious alleles (Crow and Kimura, 1970). Therefore, it determines the effect of both inbreeding depression and genetic drift (Falconer, 1989; Caballero, 1994; Nomura, 2002). Genetic drift influences the level of genetic variation. In both spawning experiments of 2003 and 2005, the effective population size was approximately half of what is expected in an idealized population under random mating ($N_e/N < 0.5$) (Table 1) suggesting the potential for rapid losses of gene diversity. These low N_e equated to an inbreeding rate of 3.0 - 4.4% which is up to four times the level of inbreeding acceptable for breeding programmes (1%: Bijma, 2000). This confirms the need to increase the N_e in the population, in order to avoid the risks of inbreeding in selection programmes that depend on mass spawning for fry production. Several methods can be employed to increase the effective population size. For a fixed number of parents, the most effective way to increase the N_e would be to reduce the variance in reproductive success by equalizing the contribution of males and females to the next generation. In practice, this might be difficult in mass spawning conditions where there is no control on mate selection, mating of the animals and when there is no marker information to reconstruct pedigree. Results in Chapter 2 and 4 showed that variation in individual parental contribution was high, the male reproductive variance being higher than that of females by several magnitudes. This variation had a large influence on effective population size. Therefore, any strategy to reduce reproductive variance can be considered as a feasible option to increase the effective population size in mass spawning. In *O. niloticus* females spawn asynchronously which leads to a higher male-male competition and lower N_e. Synchronization of females, therefore, might lead to less male-male competition and this reduced competition might increase the participation of more males in fertilizing eggs. Synchronization of the ovarian cycle in *O. niloticus* females is difficult but the problem can be alleviated through some manipulations. Little et al. (1993) suggested that conditioning of tilapia females at high density prior to their stocking in spawning tanks improves spawning synchrony and intensity. Spawning data of females in our study also showed that some females spawned up to four times, while some females fail to contribute during the spawning period. This together with the wide range of fecundity among females can lead to unequal contribution and hence might cause a reduction in effective population sizes. This can be prevented by removing brooding females from breeding tanks and taking equal number of fry from each brooding female. This intervention strategy might result in less number of fry being produced but it is feasible as the primary objective for a breeding nucleus is capturing more genetic variation than producing a larger number of fry.

Another routinely used diversity conserving practice is to increase the effective population size by spawning as many fish as possible. This option entails more economic costs because of larger space requirements, logistics, acquiring and maintenance of broodstock, but it pays off in terms of capturing higher level of genetic diversity which will in turn result in higher genetic progress. Gjerde and Rye (1998) reported that the high fecundity and higher broodstock number make it possible to have a broad long term breeding objective in the nucleus, while at the same time applying selection for a single or a few traits when producing grow out animals and thus reducing the conflict between short term and long term breeding objectives as seen in breeding programmes for livestock species.

With the total number of individuals fixed, sex ratio is the factor having the greatest potential in affecting the effective population size and over which we have most control. Theoretically, N_e is maximum when the number of males and females is equal ($N_e = 4N_{em}N_{ef} \big/ (N_{em} + N_{ef})$). In the present study, mass spawnings were conducted with three different sex ratios and the predicted level of inbreeding in each sex ratio is shown in Table 1. The sex ratio 1 was expected to result in a lower level of inbreeding but as shown in Table 1 the inbreeding associated with it is even higher than in the sex ratio 2, probably due to the high male density and higher male-male competition in sex ratio 1 leading to relatively lower male participation and higher reproductive variance. Considering both seed production and associated level of inbreeding, sex ratio 2 seems to be a more viable option for a breeding scheme based on mass spawning.

Apart from the traditional manipulations of increasing effective population size mentioned above, genetic markers could be utilized in increasing N_e of small populations. Several approaches such as frequency dependent selection and selection for heterozygosity at marker loci have been proposed as strategy to increasing N_e (Wang, 2001). The efficiency of these methods, however, has been rather low because of the limited marker information and their chromosomal distributions in many species (Wang, 2001). In *Oreochromis spp*., a second-generation genetic linkage map has been constructed and this will enable mapping and selective breeding of quantitative traits (Lee et al., 2005).

Marker information could also be used in parentage analysis; and knowledge on parentage could be employed in implementation of a two stage selection scheme that combines walk-back and optimum contribution selection proposed by Sonesson (2005) which makes it possible to achieve high genetic gains at a constrained rate of inbreeding. The first step involves selection of a batch of phenotypically superior fish and in the second stage, optimum contribution selection is used. By implementing such a scheme, the unbalanced contribution and higher rates of inbreeding in tilapia mass selection schemes can be prevented and thus results in higher genetic gains. The results show that genotyping of two batches of 50 fish were often necessary and could capture 76-92% of the overall genetic level achieved by typing all fish. The added advantage of such a scheme is, therefore, that the genotyping costs are low and that there is no common environmental effect as fish are communally reared in one big holding facility (Sonesson, 2005).

Population subdivision and exchange of breeders

In addition to strategies designed to increase effective population size, gene diversity could be maintained by subdividing a captive population into subpopulations (Kimura and Crow, 1963). Each subpopulation will lose genetic variation (i.e. heterozygosity and alleles) at a rate proportional to the effective size of that subpopulation but different subpopulations may be fixed for different alleles. Crossing of groups and transfer of animals between such groups mitigates genetic drift and will result in maintenance of more genetic diversity in the total captive population (Hedrick and Gilpin, 1997). Pooling of a group of isolated sub-populations has been shown to maintain more genetic diversity and higher reproductive fitness than a single large population of the same total size (Margan et al., 1998). Creation of sublines and selecting within each subline has also been successfully implemented in dairy cattle breeding programmes. Terawaki et al. (1998) simulated genetic selection in a closed nucleus herd subdivided into two lines and with varying degree of crossing between the two lines. After ten generations of selection, the scheme that crossed the two sublines every generation resulted in the lowest level of inbreeding, albeit with a slight decrease in genetic gain. Such schemes could also be adopted in the selective breeding of tilapia.

Given the information on the number of parents leaving offspring to the next generation, the strategy ‘selection within cohorts and exchange of breeders’ as suggested by Eknath (1991) for Indian carp and implemented by McPhee et al. (2004) for weight selection in redclaw crayfish (*Cherax quadricarinatus*) could also be implemented by tilapia breeding programmes. When the number of parents contributing to the next generation is known, the rate of inbreeding per generation can be calculated using the formula of Falconer and Mackay (1996)

$$\Delta F = \frac{1}{8f} + \frac{1}{8m}$$

Where ΔF is the average rate of inbreeding per generation; f and m are the number of females and males leaving progeny to the next generation.

Taking the mass spawning conducted in 2003 as an example, the effective number of males and females contributing to the next generation were as follows: Hapa A (N_{em} = 5, N_{ef} = 18) and hapa B (N_{em} = 5, N_{ef} = 16) (Table 1). These two experimental breeding hapas can be considered as model broodstock units, and the level of inbreeding per generation for different number of breeding units can be calculated (Figure 1). For example, assuming a similar number of contributing males and females as in the experimental breeding units, three units of breeders will result in a level of inbreeding of 1.06% per generation which is very close to the acceptable level of inbreeding for most breeding programmes. For small scale farmer-based breeding programme, three breeding units as our experimental spawning stock would have a census population size of 111 adults (= 37*3; 25 females and 12 males per cohort) and N_e of 56 (= 111*0.5) spawning adults should be sufficient to meet the constrained inbreeding of 1%. In conservation management, on the other hand, there is a generally accepted view that a minimum effective population size of 50 spawning individuals is required to capture genetic diversity that will sustain the short-term fitness of a population (Allendorf and Ryman, 2002). Using a $N_e/N \approx 0.5$ from our study, this translates into a census population size of 100. Therefore, from both the rate of inbreeding per generation and the conservation rule of thumb, three breeding units of broodstock can be recommended as a founder population for a small-scale breeding scheme run by an individual farmer. Nucleus breeding schemes offer the potential for higher genetic progress than would be achieved by traditional within-farm selection (Smith, 1988). The rate of genetic progress depends, among other things, on the size of the nucleus, selection intensity and the effectiveness of selection within the nucleus (Weigel, 2001). The experimental breeding units can be considered as building blocks and the reproductive parameters might be extrapolated to design bigger schemes. In general for faster rates of genetic progress in a nucleus, higher population size and higher selection intensities are required. In case a bigger population in a nucleus would pose an economic challenge, structured exchange between smaller nucleuses could still be effective provided that the schemes have similar population size, selection criteria and goals.

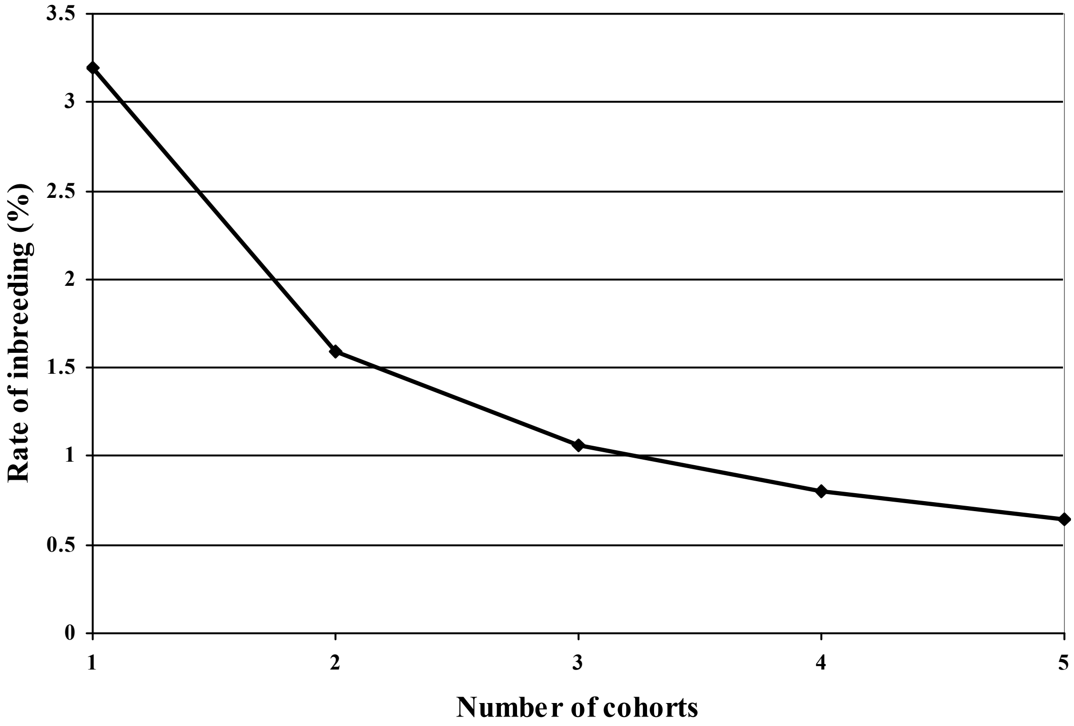

Figure 1. Number of broodstock units (cohorts) and associated level of inbreeding per generation

Exchange of breeders between breeding units in a farm or between nucleus breeding schemes can be achieved by selecting the best males in one unit and then shifting them to another cohort in a cyclical mating system as described by Cockerham (1970). The cyclical mating systems have been successfully implemented in Asian breeds of Nile tilapia through within-family selection and results have shown that the average rates of inbreeding of 1.4% per generation in the stocks selected for twelve generations were smaller than what is typically observed in cultured populations (Bolivar and Newkirk, 2000). In contrast to single pair matings, selection within cohorts with exchange of breeders between cohorts following a prescribed pattern is a feasible design even with limited resources. Field personnel find it easy to follow and can thus adhere to instructions provided (Ponzoni et al., 2006). In populations under selection, avoiding loss of desirable genetic diversity may be impossible, but the problem of inbreeding in aquaculture stocks can be forestalled by exchange of breeders or by keeping high effective populations sizes. It should be emphasized that subdividing the population or exchange of breeders does nothing to reduce the level of inbreeding in the base population, but it serves only to reduce the rate at which further inbreeding is accumulated. Therefore, it is essential that a relatively high level of genetic diversity should be present in the starting broodstock.

Implications for natural/semi-natural populations

The amount of genetic diversity in natural populations is the basis of adaptability to environmental changes and evolutionary potential of species. The ability of natural populations to maintain genetic variation depends not only on the number of individuals constituting a population, but also on the mating systems of the species. Quantitative estimates of mating system parameters in fish populations are, therefore, necessary to explain the genetic structure of these populations and to predict adaptability and evolutionary processes.

Multiple paternity, effective population size and inbreeding

Numerous studies have shown that multiple paternity within a single reproductive bout (e.g., clutch, litter, nest) is widespread among species with external fertilization and parental care (Avise et al., 2002 and references therein). Microsatellite-based paternity analyses in several cichlids have documented multiple paternity of broods, with up to six males fertilizing a single clutch (Kellogg et al., 1995; Parker and Kornfield, 1996). In our study, the prevalence of multiple paternity was high: 64% (sex ratio 1), 43% and 51% (sex ratio 2), 32% (sex ratio 3) of spawnings were multiply sired. Up to five males were involved in fertilizing eggs in a single spawn (Chapter 2 and Chapter 4). Multiple paternity, compared to single paternity, can influence effective population size (Sugg and Chesser, 1994) and the reproductive efficiency of individuals (Levitan and Sewell, 1998). This mating system has been advocated as a strategy to reduce inbreeding (Stockley et al., 1993) and maintain genetic diversity in small natural populations. Broadly, multiple paternity has been claimed to provide genetic benefits by improving the chance that females will acquire ‘‘good’’ genes or enhancing the genetic diversity of their progeny (Yasui, 1998). Moran and Garcia-varquez (1998) observed that multiple paternity was a way to increase the effective population size and consequently to maintain genetic variability of the relict Atlantic salmon population in river Mandeo (La Coruña, Spain) which otherwise would have suffered higher levels of inbreeding due to very low number of adults and might have faced extinction. In their study, complete male dominance was not observed and no more than 28% of the total progeny was fertilized by a single male. In contrast to Moran and Garcia-varquez (1998), the relative contributions of males in most of the multiply-sired spawns in our study were characterized by one single dominant male siring most of the offspring and other males with only minor contributions. This very high variance in reproductive success and involvement of only few males in most of the spawns resulted in reduction of effective population size and increased rate of inbreeding compared to an idealized population under random mating. Furthermore, there were no significant differences in the hatching rates of both the single pair spawnings and mutiply-sired spawns suggesting that direct benefits of multiple paternity could not be discerned from our data. Overall, our data did not reveal any of the claimed benefits of multiple paternity. The high reproductive skew, dominance of few males and the associated level of inbreeding might have masked the potential advantages of multiple paternity.

The N_e/N in our study ranged from 0.3 to 0.44 indicating that less than 50% of the parents were contributing to the gene pool of the next generation. In natural populations, low N_e/N ratios (e.g. $N_e/N \approx 0.10$; Frankham 1995a) are common and have been attributed to the exposure of natural populations to natural stressors such as weather extremes, high population densities, parasites and diseases (Young 1994; Pray et al., 1996). These natural factors promote population size fluctuations and large variation in family sizes that will eventually reduce N_e/N (Frankham, 1995a). Whether inbreeding affects the demography and persistence of natural populations has been questioned. There are evidences that inbreeding depression can occur and can decrease the fitness of wild populations (e.g. Spielman et al., 2004; Reed, 2005). However, some other studies of natural populations have yielded no evidence of inbreeding depression, despite small population size or genetic homogeneity (Visscher et al., 2001; Kalinowski et al., 1999). The finding in this thesis (Chapter 4) showed that inbreeding decreased the reproductive fitness of both males and females: inbred adults had lower reproductive success compared to outbred individuals and that inbreeding depression on male reproductive success was magnified under higher male density. The higher reproductive success of males could be due to the advantage of outbred/better conditioned males in male-male competition or it could also be active mate choice by females since body size and condition fish are some of the cues used by females during mate choice (Houde, 1997). However, given our experimental setup, it is not possible to disentangle effects of male-male competition and active mate choice by females. But clearly the results reveal the presence of a natural selective force that favours outbred adults and uncovers a mechanism that counterbalances the accumulation of inbreeding to some extent in populations under natural mating systems.

A number of other strategies for reducing inbreeding accumulation such as avoidance of mating with close kin (Brown and Eklund, 1994) and natal dispersal (Pusey, 1987) have been documented in many natural populations. Results in Chapter 4 did not reveal any effect of relatedness (as measured by the kinship coefficient) on the reproductive success of males suggesting there was no kin recognition and avoidance. Our experimental breeding hapas allow natural mating but the crowded and artificial conditions might not be an appropriate milieu for studying natural mechanisms of inbreeding avoidance which could be greatly influenced by the nature of the environment. For example, *Sarotherodon melanotheron* samples originating from lagoon areas tended to show significant heterozygosity deficiencies as compared with Hardy-Weinberg equilibrium expectations, whereas this was not the case for riverine samples. This is because in lagoon type of environment where the water hydrodynamicity is much lower than within rivers, allowing mating within small group of kin as compared to rivers where there is higher chance for dispersal (Pouyaud et al., 1999). In Lake Malawi cichlids, male-biased within-population dispersal has been noticed explaining the absence of inbreeding despite the very no /or low dispersal between population occupying adjacent areas (Knight et al., 1999). In populations of *Oreochromis niloticus*, these mechanisms of inbreeding avoidance might be at work and it is worthwhile investigating them in a natural system or in an experimental set up more close to natural environment.

Conclusions

Mass selection breeding schemes which depend on mass spawning for fry production can present a straightforward and economically viable option for low-cost genetic improvement programmes. However, many mass selection schemes in fish have not lead to satisfactory results because high selection intensities often lead to excessive inbreeding over time and resulted in no or marginal genetic gain. The results of this thesis have established the effective number of parents contributing to the next generation and the associated level of inbreeding per generation in experimental mass spawning broodstock units (breeding units). Several traditional strategies as well as marker-assisted manipulations have been discussed as a possibility of increasing the effective population sizes or forestalling the rate at which genetic diversity is lost. In addition, a critical size ratio for cannibalism has been established to develop a collimation procedure which will reduce the phenotypic variance in growth that can be caused by size-dependent competition and cannibalism among fish. The information presented in this thesis is thus a combination of strategies to increase genetic diversity, reduce level of inbreeding and minimize the non-genetic phenotypic variance. Such information can be used to develop practical scenarios for mass selection breeding schemes that maximise selection response while at the same time managing inbreeding. Considering the breeding units as basic building blocks, mass selection breeding programmes based on mass spawning can be implemented. The number of breeding units can be extrapolated depending on the size of the nucleus and the intensity of selection.

In conclusion, the results in this thesis have laid the ground work for a mass selection scheme that takes into consideration the genetic contribution of parents under mass spawning mating conditions and management of ΔF. Such schemes require minimum infrastructural investments and can be adopted as a model for low-cost and effective selective breeding schemes that will result in desired response without compromising the long term fitness of culture populations due to inbreeding.

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Summary

Summary

Nile tilapia (*Oreochromis niloticus* L.) is one of the most important species among the commercially farmed tilapias. Both small-scale and commercial production of tilapia is rapidly expanding in many countries of the world because tilapias meet many of the desirable characteristics for an ideal species for aquaculture: fast growth, high acceptability to consumers and tolerance of a wide range of culture conditions. Fry production in Nile tilapia, *Oreochromis niloticus* is typically achieved by mass-spawning of males and females stocked in large hapas, earthen ponds or tanks. Territorial behaviour and reproductive competition in such spawning setup together with the asynchronous spawning nature in tilapia could have serious implications for breeding plans that employ mass spawning for seed production. The aims of this thesis were to gain thorough understanding into the natural mating systems in *O. niloticus*; the genetic consequences of the mating system (reproductive success, effective population size and inbreeding); as well as non-genetic variations (size heterogeneity and cannibalism) among fish as a result of asynchronous reproduction.

In order to establish captive breeding programmes that maximize genetic gain without compromising the long-term genetic diversity of stocks, understanding of the natural mating systems is a crucial step. In **Chapter 2**, we analyzed parentage and quantified male reproductive success of *O. niloticus* under commercial hatchery conditions in two breeding hapas each stocked with 12 males and 25 females by sampling offspring from individual mass-spawning events. Eleven highly polymorphic microsatellite markers were used to genetically characterize the mating systems in *O. niloticus* and quantify the reproductive success of individual males and females. In total, 760 offspring from 76 spawnings were included in the paternity analysis. Mating system in Nile tilapia ranged from single pair to promiscuous mating. Multiple paternity was detected in 46% of the broods, with up to 4 males fertilizing a single clutch. Multiple maternity was also detected in over 8% of the clutches analyzed. There was a very high variance in male reproductive success, with one third of males siring more than 70% of the offspring. Male condition factor had a significant effect on reproductive success with better conditioned males siring a large proportion of offspring. The high reproductive variance resulted in a N_e of around 16 for hapas A and B, and a low N_e/N ratio, 0.43 and 0.45 for hapas A and B respectively. The rate of inbreeding, ΔF, for each hapa, was estimated to be around 3.0% per generation, which is about twice the inbreeding expected in an idealized population of the same census size. When designing of

fish breeding programmes based on mass-spawning, the higher inbreeding and lower effective population size should be taken into consideration.

In mating systems with skewed reproductive success, the effective population size (N_e) of a population becomes smaller. As a result, small populations experience an increased degree of inbreeding (ΔF). Inbreeding depression in fish affects fitness related traits such as survival, growth, reproductive traits and other morphological traits. The effect of genetic depression on different traits in *O. niloticus* was investigated in **Chapter 3**. Experimental fish were produced in a full-sib/half-sib mating design in which each sire was mated to two dams and each dam mated to only one sire. We mated 20 sires and 35 dams to produce 35 full-sib families with expected inbreeding coefficients (F) of 0.0, 6.3, 9.4, 12.5 and 25%. The fry were produced and reared in hapas suspended in fertilized ponds until time of tagging. In total 1832 fish were stocked in two fertilized earthen ponds for a period of 8 months (including 3 months of over-wintering). At the end of the experiment 389 fish were harvested. Results show that level of inbreeding significantly affected early fry survival and body weight at stocking. Level of inbreeding did not have significant effect on both the pond survival and weight at harvest. Effects of pond, weight at stocking, sire and dam component significantly affected body weight at harvest and pond survival. Fluctuating asymmetry was significantly affected by the dam component and pond but not by the level of inbreeding. This suggests that FA was more influenced by environmental factors than by genetic factors. The results of this study emphasizes that inbreeding had a significant effect on survival and growth on early but not on later stage of development. The observed difference in effects of inbreeding on early and later life stages can be explained by strong natural selection on fitness during over-wintering.

Inbred individuals that survive the selective pressure to adulthood might still suffer reduced adult survival and reproductive success. **Chapter 4** investigated the effects of inbreeding on reproductive success in Nile tilapia, *O. niloticus* under semi-natural conditions in breeding hapas. A total of 72 fish (27 males and 45 females) from nine full-sib families (8 fish/family) were divided into two and stocked in two hapas at a sex ratio of 1 and 3. Fish had inbreeding coefficient of F = 0.0, 6.3, 9.4 and 25%. Fry were collected from the mouth of incubating females on a weekly basis and parentage assignment was done on 1120 offspring from 56 spawnings using seven polymorphic microsatellite markers. Female reproductive output (fecundity), expressed as the total number of eggs per spawning, was significantly affected by the level of inbreeding and body weight. Per 10% increase of the level of inbreeding, egg number declined by 11% of the mean. Male reproductive success, calculated as the proportion of offspring sired per spawning, was affected by the level of inbreeding, condition factor, sex ratio and gonad weight of males. Per 10% increase in the level of inbreeding, male reproductive success declined by 40%. The decline in reproductive success was higher in sex ratio 1 compared to sex ratio 3 indicating that the inbreeding depression was magnified under higher male-male competition. Degree of relatedness between mating pairs (Kinship coefficient), however, did not have any effect on reproductive success of males. Overall, our findings show that inbred individuals have lower reproductive success and uncover a natural selective pressure that favors outbred males and that counterbalances accumulation of inbreeding.

Asynchronous nature of reproductive cycles of individual broodstock in *O. niloticus* leads to episodic fry production. This presents a problem to aquaculture in two ways: 1) massive fry losses due to size-dependent cannibalism; 2) mixed age/size populations and associated cannibalism can mask the genetic merit of individuals. Fish breeders practising mass selection might inadvertently select for cannibalism as large size, faster growth rate and robustness are those phenotypic traits which a breeder desires in the selected broodstock. Selection under such circumstances can mask genetic merit and obscure selection response in mass selection programmes. In order to develop a protocol for size grading and minimize such effects, factors influencing size-dependent cannibalism in Nile tilapia, *Oreochromis niloticus*, were investigated in controlled conditions (**Chapter 5**). First, individual-based trials were conducted to develop a linear regression model to predict the occurrence of cannibalism based on body measurements. Oral gape (G, mm), body depth (D, mm) and weight (W, g) of 140 fish were measured and we estimated maximum prey weight (W_{prey}) for a given predator weight ($W_{predator}$): $Log10Wprey = 1.03Log10W_{predator}-1.13$. This model was verified by conducting 76 single pair trials. The revised model based on observed cannibalism is: $Log10W_{prey} = Log10W_{predator}-1.17$. Several experiments involving a group of prey and predators were conducted to elucidate the relative importance of factors that influence cannibalism. Cannibalism was significantly affected by stocking density, age of fish and predator/prey weight ratio. Logit models of mortality data of both the individual-based and observations based on a group of predators and prey estimated R_{50} (the predator/prey weight ratio at which an encounter of a predator and prey resulted in a predation success of 50%) to be 14.6 and 14.8, respectively. The results obtained in this experiment can be used to derive simple management of heterogeneous fry groups (e.g. grading of fish). Grading will have implications for reducing fry losses, minimizing the non-genetic variations caused by size dependent cannibalism among fish and can improve the efficiency of mass selection.

The results described in this thesis are discussed in **Chapter 6.** This study demonstrated that a thorough understanding of mating systems and reproduction parameters are crucial for outlining strategies that ensure the maintenance of genetic diversity in breeding schemes employing mass spawning for fry production. With the knowledge of mating systems and the number of parents contributing to the next generation, mass selection based on mass spawning can be used as a model for low cost breeding schemes that ensure a desired level of genetic gain without compromising the genetic diversity of the stocks due to inbreeding. In addition, knowledge on mating systems and other population parameters can be used in the management of supportive breeding programmes for restoration of endangered wild fish populations.

Samenvatting

Samenvatting

Nijl tilapia (*Oreochromis niloticus* L.) is de belangrijkste soort onder de commercieel gekweekte tilapias. Wereldwijd nemen zowel de grootschalige als kleinschalige teelt zeer snel toe, vooral vanwege de ideale eigenschappen van Nijl tilapia als aquacultuur soort, zoals snelle groei, hoge waardering door consumenten, en tolerantie tegen een grote verscheidenheid aan kweekomstandigheden.

Productie van Nijl tilapia broed vindt plaats door ouderdieren bij elkaar in aarden vijvers, tanks of hapas te plaatsen. De voortplanting is geheel natuurlijk en normaal kunnen binnen 20 dagen na het bij elkaar plaatsen van de ouders de vislarven geoogst worden. Territoriaal gedrag en competitie van mannetjes om vrouwtjes, en niet synchrone voortplanting van vrouwtjes kunnen echter grote consequenties hebben voor fokprogramma's waarin deze manier van voortplanten wordt gebruikt. Het doel van het in dit proefschrift beschreven onderzoek was om een goed begrip te krijgen van het natuurlijke voortplantingssysteem van *O. niloticus.* Hierbij lag de nadruk op de genetische consequenties van het voortplantingssysteem, zoals reproductief succes, effectieve populatie grootte en inteelt. Daarnaast is ook gekeken naar de gevolgen van a-synchroon voortplanten voor heterogeniteit in grootte en kannibalisme.

Een goed begrip van het natuurlijke voortplantingssysteem is van cruciaal belang voor het ontwerpen van fokprogramma's waarbij genetische vooruitgang gemaximaliseerd wordt zonder dat de genetische diversiteit op de lange termijn in gevaar komt. Vrouwelijke Nijl tilapia incuberen de bevruchte eieren in de bek tot de larven uitkomen. In **hoofdstuk 2** worden afstamming en mannelijk reproductief succes onder commerciële omstandigheden gekwantificeerd door larven uit de bek van vrouwtjes te verzamelen uit 2 hapas die elk 12 mannetjes en 25 vrouwtjes bevatten. Elf polymorfe microsatelliet merkers werden gebruikt om de afstamming van elk van de larven te bepalen. In totaal werden 760 nakomelingen uit 76 paringen gebruikt in de afstamminganalyse. Voortplantingspatronen varieerden van monogame paringen tot promiscue paringen. Paternale polygamie trad op in 46% van de verzamelde groepjes eieren, waarbij soms tot 4 vaders betrokken waren bij het bevruchten van de eieren van een enkel vrouwtje. Maternale polygamie trad op in meer dan 8% van de verzamelde ei groepjes. Er was een grote variatie in mannelijk reproductief succes, waarbij een derde van alle mannetjes de vader waren van meer dan 70% van de nakomelingen. Mannelijke conditie factor had een significant effect op reproductief succes waarbij mannetjes

met een hogere conditiefactor meer nakomelingen hadden. De effectieve populatiegrootte N_e was ongeveer 16 voor beide hapas, met een lage N_e/N ratio van 0.43 en 0.45 voor respectievelijk hapa A en B. De toename van inteelt, ΔF, voor elke hapa werd geschat op ongeveer 3% per generatie, wat twee keer de toename van inteelt is van een ideale populatie met vergelijkbare omvang. Bij het ontwerp van vis fokprogramma's gebaseerd op natuurlijke voortplanting, dient rekening gehouden te worden met de hogere inteelt en kleinere effectieve populatiegrootte.

Wanneer het individuele voortplantingssucces scheef verdeeld is wordt de effectieve populatiegrootte Ne kleiner. Dit betekent dat kleinere populaties een verhoogde toename van inteelt ondergaan. Inteelt depressie in vissen uit zich in fitness gerelateerde kenmerken zoals overleving, groei, reproductie en in morfologische kenmerken. In **hoofdstuk 3** werd gekeken naar het effect van inteelt op diverse kenmerken in *O. niloticus*. Experimentele vissen werden geproduceerd door elk mannetje met 2 vrouwtjes te paren, en elk vrouwtje maar met een mannetje. In totaal werden 20 mannetjes en 35 vrouwtjes gepaard om 35 full-sib families te produceren met inteelt coëfficiënten (F) van respectievelijk 0, 6,3, 9,4, 12,5 en 25%. De larven werden geproduceerd en opgekweekt in hapas in bemeste vijvers tot dat ze gemerkt konden worden. In totaal werden 1832 vissen gemerkt en opgekweekt in bemeste vijvers gedurende een periode van 8 maanden (waarvan 3 gedurende de winter). Aan het einde van het experiment werden 389 vissen geoogst. Het niveau van inteelt had een significant effect op vroege larvale overleving en lichaamsgewicht op het moment van merken. Inteelt had geen effect op vijveroverleving en gewicht na 8 maanden. Er waren significante effecten van vijver, gewicht bij merken, en vader en moeder componenten op lichaamsgewicht na 8 maanden en overleving. Fluctuerende asymmetrie (FA) werd beïnvloed door de moeder en de vijver waarin de vissen opgroeiden, maar niet door inteelt. Dit suggereert dat FA meer door milieu componenten dan door genetische factoren beïnvloed wordt. De resultaten van deze studie benadrukken dat inteelt een significant effect op overleving en groei op vroege leeftijd van tilapia heeft. Het verschil in effect op vroege en late leeftijd kan worden verklaard door een sterke natuurlijke selectie op fitness gedurende de overwintering.

Ingeteelde dieren die toch overleven en volwassen worden kunnen nog steeds last hebben van verminderd reproductief succes. In **hoofdstuk 4** worden de effecten van inteelt op reproductief succes onderzocht onder semi-natuurlijke condities in hapas. Een groep van 72 vissen (27 mannetjes en 45 vrouwtjes) uit 9 full-sib families (8 vissen per familie) werden verdeeld in 2 groepen en in hapas geplaatst in een sex ratio van 1:1 of 1:3. De inteeltcoefficient F van de vissen was 0, 6,3, 9,4 of 25%. Broed werd wekelijks verzameld uit de bek van de vrouwtjes. Afstamming werd bepaald van 1120 larven uit 56 paringen, met behulp van 7 microsatelliet merkers. Vrouwelijke fecunditeit, uitgedrukt als het totale aantal eieren per paring, werd significant beïnvloed door lichaamsgewicht en de mate van inteelt. Met elke 10% toename in teelt neemt de fecunditeit met 11% af. Mannelijk reproductief succes, berekend als proportioneel aandeel per paring, werd beïnvloed door inteelt, conditiefactor, sex ratio en testis gewicht van mannetjes. Met elke 10% toename in inteelt nam het reproductief succes van mannetjes af met 40%. De afname was groter bij een sex ratio van 1:1 dan bij een sex ratio van 1:3. Dit impliceert dat de effecten van inteeltdepressie vergroot werden bij intense competitie van mannetjes onderling. De mate van verwantschap tussen potentiële ouders had geen invloed op het uiteindelijke reproductieve succes van mannetjes. Dit betekend dat inteelt niet vermeden wordt via paarkeuze, maar dat inteelt op populatieniveau wel sterk beperkt wordt door verminderd reproductief succes van ingeteelde mannetjes.

Als voortplanting cycli van *O. niloticus* vrouwtjes niet synchroon verlopen leidt dit tot pieken en dalen in de productie van nakomelingen. Voor de aquacultuur is dit een probleem, om twee redenen: 1) initiële verschillen in grootte en leeftijd van larven kunnen vergroot worden door kannibalisme, en 2) in qua leeftijd gemengde populaties is het moeilijk om de genetische waarde van individuen op basis van eigen prestatie te schatten. Fokkers die selecteren op eigen prestatie kunnen zo onbedoeld kannibalistische vissen selecteren aangezien grootte, groeisnelheid en robuustheid fokdoelen zijn waarop bij voorkeur geselecteerd wordt. Om deze onbedoelde effecten te minimaliseren is het nodig om te sorteren om zo de grootte verschillen in de populatie te structureren. Met de uiteindelijke bedoeling een protocol voor sorteren te ontwikkelen werd een experiment opgezet waarbij het optreden van kannibalisme onder gecontroleerde condities werd bestudeerd (**hoofdstuk 5**). Eerst werd een lineair regressie model ontwikkeld om het optreden van kannibalisme te voorspellen op basis van lichaamsafmetingen van predators (kannibalen) en prooien. Bekgrootte (G, mm), lichaamshoogte (D, mm) en gewicht (W, g) van 140 vissen werden gemeten. De maximum prooi grootte (W_{prooi}) voor een gegeven predator gewicht (P_{predator}) werd geschat als: $\text{Log10Wprooi} = 1.03\text{Log10W}_{\text{predator}}-1.13$. Dit model werd geverifieerd in 76 een-op-een confrontaties. Het aangepaste model op basis van de geobserveerde kannibalisme is: $\text{Log10W}_{\text{prooi}} = \text{Log10W}_{\text{predator}}-1.17$. Vervolgens werden verschillende experimenten met

groepen van prooien en predatoren uitgevoerd om de relatieve belangrijkheid van diverse factoren die kannibalisme beïnvloeden te onderzoeken. Kannibalisme werd significant beïnvloed door bezettingsdichtheid, leeftijd van de vis en de predator/prooi ratio. Logit modellen van mortaliteit data van individuele- en groepsconfrontaties werden gebruikt om de R_{50} (de predator/prooi gewicht ratio waarbij in 50% van de gevallen kannibalisme optreed) te schatten. Deze was 14.6 voor individuele confrontaties en 14.8 voor groepskannibalisme.
De resultaten van dit experiment kunnen worden gebruikt om eenvoudige protocollen te ontwerpen om kannibalisme in heterogene larvengroepen te verminderen, bijv. door te sorteren. Sorteren zal kannibalisme verminderen, de niet-genetische variatie verminderen en de efficiëntie van selectie op basis van eigen prestatie verbeteren.

De resultaten beschreven in dit proefschrift worden bediscussieerd in **hoofdstuk 6.** Deze studie demonstreert dat een grondig begrip van paringsgedrag en reproductie parameters cruciaal zijn voor het ontwikkelen van strategieën, die bij fokprogramma's waarbij natuurlijke voortplanting wordt gebruikt, het behoud van genetische diversiteit verzekeren. Met de kennis van paringsgedrag en het aantal ouders dat bijdraagt aan de volgende generatie kan een goedkoop massaselectie programma, gebaseerd op natuurlijke voortplanting, worden ontworpen waarbij genetische vooruitgang gemaximaliseerd zonder dat er ongewenste inteelt optreed. Tevens kunnen kennis over paringsgedrag en andere populatie parameters gebruikt worden in het beheer van ondersteunende fokprogramma's voor het herstel van bedreigde vispopulaties.

Acknowledgements

This study would not have been possible without the support and encouragement of many individuals. I am grateful to my promtors Prof. Johan van Arendonk and Prof. Johan Verreth for their constructive criticism and support. My foremost gratitude goes to my supervisors Dr. Hans Komen and Henk Bovenhuis for their guidance and enlightening discussions. Your enthusiasm and prompt responses were extraordinary! A special thanks for that. I would also like to thank Dr. Mahmoud Rezk for his practical help in the field and Dr. Richard Crooijmans for his guidance in the molecular analysis.

I would like to thank all my colleagues and friends at both the Animal Breeding & Genetics group and Aquaculture & Fisheries group. During my stay I was fortunate to share office with many colleagues: Harrison, Marc, Bosso, Solomon, Brigitte, Robbert and Mikhail- thanks for all the nice discussions. Special thanks are due to many friends and colleagues: Catarina, Oliver, Iyob, Guenzuelo, Paula, Miriam, Samad, Pascal, Patricia, Mohamed Ali, An, Titu, Jascha. I really enjoyed the ambiance of friendship, collegiality and many exotic dinners - Thank you all!

Special thanks go to many organizations which provided financial supported for my PhD project. I am indebted to NUFFIC, University of Asmara, Wageningen University and the WorldFish Center for providing me funds and necessary facilities. Special thanks to Dr. Hans Komen for his relentless effort to make this project financially viable. The staff of the WorldFish Center, Egypt is acknowledged for facilitation of my work and making my stay in Abbassa comfortable. Many thanks to Roel Bosma, your help as a project manager is highly appreciated.

I am also grateful to students who contributed to this project: Anamul Kabir, Zizy El-Bialy and Ugur Ozturk. Thank your for your help and I wish you all the best in your future endeavors.

Life would have been difficult without friends in the Netherlands: Iyob, Sammy G, Sammy P, Mike (Berari keyat), Danny, Abraham, Abeba, Tamrat, Hideat, Suleiman, Debby – Thanks you all for the nice and momentous time we had together. I would also like to extend my gratitude to my family in the Netherlands Ghenet, Aklilu, Abeba, Hideat and her family.

Special thanks to my friends from Iowa Elizabeth and Charles Sukup – thanks for your many visits to Wageningen. My long time friends from Ghent deserve special appreciation: Mariska, Katrien, Solomon, Lieve and Yonas –you were there for me whenever I needed your help and thank you all.

My deepest gratitude goes to all my family in Eritrea. My mother and father, Fessehaye and Ghidey thanks for your love, dedication and unwavering support at all times. I also appreciate all the support and encouragement from all my sisters and brother. Special thanks are due to Brkti and Taddesse

My dearest Tsega, words won't be enough to express my gratitude for your love, unflagging support, dedication and optimism. You were my rock and your thoughts propelled me during difficult times. For this I will be eternally grateful. Thank you Tsegi - You are the best!!!!

List of Publications

Peer-Reviewed papers

Fessehaye, **Y**., El-bialy, Z., Rezk, M.A., Crooijmans, R., Bovenhuis, H., Komen, H., 2006. Mating systems and male reproductive success in Nile tilapia (*Oreochromis niloticus*) in breeding hapas: A microsatellite analysis. *Aquaculture* 256, 148-158

Fessehaye, **Y**., kabir, A., Bovenhuis, H., Komen, H., 2006. Prediction of cannibalism in Juvenile Nile Tilapia, *Oreochromis niloticus* based on predator to prey weight ratio; and effects of age and stocking density. *Aquaculture* 255, 314-322.

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Fessehaye, **Y**., Komen, H., Rezk, M.A., van Arendonk, J.A.M., Bovenhuis, H. Effects of inbreeding on survival, body weight and fluctuating asymmetry (FA) in Nile tilapia, *Oreochromis niloticus*. *Aquaculture* (Accepted).

Viveiros, A.T.M., **Fessehaye, Y.**, Ter Veld, M., Schulz, R.W., Komen, J., 2002. Hand-stripping of semen and semen quality after maturational hormone treatments, in African catfish *Clarias gariepinus*. *Aquaculture* 213, 373-386

Fessehaye, Y., Bovenhuis, H., Rezk, M.A., Crooijmans, R., van Arendonk, J.A.M., Komen. H., Effects of inbreeding and sex ratio on reproductive success in mass-spawning of Nile tilapia, *Oreochromis niloticus*. (to be submitted to *Molecular Ecology*)

Proceedings and Abstracts

Fessehaye, Y., Bovenhuis, H., Komen, H., 2006. Genetic quality of *Oreochromis niloticus* fry produced from mass spawnings: Threats and opportunities for small scale aquaculture and selective breeding. *In*: Fishponds in Farming Systems, 2006. Zijpp A.J. van der, J.A.J. Verreth, M.E.F. van Mensvoort, R.H. Bosma, M.C.M. Beveridge (Eds.). Proceedings of INREF-POND symposium, Can Tho, Vietnam.

Fessehaye, Y., Komen, H., Rezk, M.A., Ponzoni, R.W., Bovenhuis, H., 2006. Effect of inbreeding on the performance of Nile tilapia (*Oreochromis niloticus*). Book of Abstracts, International symposium-Genetics in aquaculture IX, June 26-30, 2006 Montpellier, France.

Fessehaye, Y., Z. El-bialy, Rezk, M.A. Crooijmans, R., Bovenhuis, H., Komen, H., 2005. Microsatellite-based parentage analysis and males' reproductive success in Nile tilapia, *Oreochromis niloticus*. Book of Abstracts, World Aquaculture Society meeting in Bali, Indonesia**.**

Fessehaye, Y., Rezk, M.A., Bovenhuis, Henk and Komen, H. 2004. Size Dependent Cannibalism in Juvenile Nile tilapia (*Oreochromis niloticus*). *In*: Bolivar, R., Mair, G., Fitzsimmons, K. (Eds.), New Dimensions in Farmed Tilapia. Proceedings of the 6th International Symposium on Tilapia in Aquaculture (ISTA 6), Manila, pp.

Training and Supervision Plan **Graduate School WIAS**

Name	Yonas Fessehaye	
Group	Animal Breeding and Genetics/Aquaculture and Fisheries	
Daily supervisor(s)	Dr. Hans Komen, Dr. Henk Bovenhuis, Wageningen University Dr. Mahmoud Rezk, The worldFish Center	
Supervisor(s)	Prof. J.A.J. Verreth and Prof. J.A.M. van Arendonk	
Period	June 2002 until June 2006	
Submitted	10-09-2006	first plan/midterm/**Certificate**

The Basic Package (3 ECTS)	year	ECTS
WIAS Introduction Course	2004	1.5
Course on philosophy of science and/or ethics	2003	1.5
Subtotal Basic Package		**3.0**
Scientific Exposure (Conferences, seminars and presentation)	year	ECTS
International conferences (minimum 2 ECTS)		
International symposium on Tilapia in Aquaculture (ISTA 6), Manila, the Philippines	2004	1.2
World Aquaculture 2005, Bali, Indonesia	2005	1.2
WorldFish Center peer-review meeting, Egypt	2006	1.2
Seminars and workshops		
WIAS Science Day 2003,2004	2004	0.6
WIAS seminar "vitality in Fish", Wageningen NL	2005	0.3
Presentations		
Poster presentation WIAS Science Day 2004, 2005 Wageningen, NL	2004	1.0
Oral presentation in ISTA6, Manilla, Philippines	2004	0.5
Oral presentation in World Aquaculture 2005, Bali	2005	0.5
Oral presentation WorldFish peer-review meeting, Egypt	2006	0.5
Subtotal		**7.0**
In-Depth Studies (minimum 4 ECTS)	year	ECTS
Advanced statistics courses (optional)		
Design and Analysis of experiments	2002	1.0
Advanced Regression analysis, Center for Biostatistics, University of Utrech (28/29 September)	2006	0.6
Longitudinal data & repeated measurements, Center for Biostatistics, University of Utrech (19/20 October)	2006	0.6
Factor and cluster analysis, Center for Biostatistics, University of Utrech (2/3 November)	2006	0.6
PhD students' discussion groups (optional)		
Quantitative discussion group (Animal breeding and Genetics group)	2005/2006	1.0
Undergraduate courses (only in case of deficiencies)		
Animal breeding & Genetics	2002	4.0
Genomics	2004	4.0
Subtotal		**12.0**
Professional Skills Support Courses	year	ECTS
WIAS Course Techniques for Scientific Writing (advised)	2002	1.2
Career perspective	2005	1.5
Subtotal Professional Skills Support Courses		**3.0**
Research Skills Training (apart from carrying out the PhD project, optional)	year	
Preparing own PhD research proposal (optional, maximum 4 ECTS)		4.0
Subtotal		**4.0**
Didactic Skills Training (optional; not more than 10-15 % of your total phd time)	year	ECTS
Supervising MSc theses		
Anamul kabir	2003	1.5
Zizy El bialy	2005	1.5
Subtotal		**3.0**
Education and Training Total (minimum 30 ECTS)		**32.0**

About the Author

YONAS FESSEHAYE was born on November 28, 1972 in Asmara, Eritrea. In 1996, he received a B.Sc degree in Marine Biology from University of Asmara, Eritrea. From September 1996 till September 1998 he worked as a teaching assistant at the same department. In 2000, he obtained an M.Sc degree in Aquaculture from University of Ghent in Belgium (Laboratory of Aquaculture and Artemia Reference Center). From 2000-2002, he taught several courses at the department of Marine Biology and Fisheries, University of Asmara. In June 2002 he was awarded a scholarship to pursue his PhD research at Wageningen University.

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The research in this thesis was financed by the Netherlands organization for international cooperation in higher education (NUFFIC) via the MHO-University of Asmara project, INREF-POND of Wageningen University and the WorldFish Center.

Financial support for printing of this thesis was obtained from the Dr. Judith Zwartz foundation, Wageningen, The Netherlands and the LEB funds of Wageningen University.

Printed by: Ponsen en Looijen BV, Wageningen, The Netherlands.

Cover design: Bereket G.