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Performances of Nile Tilapia (*Oreochromis niloticus*) Selected for High Growth Rate After Three Generations

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Abstract: A selective breeding study for live weight based on a fully pedigreed population of GIFT (Genetically Improved Farmed Tilapia) strain was carried out at the Aquaculture Extension Centre, Department of Fisheries, Jitra, Kedah. Progeny were generated in three spawning seasons, 2002, 2003 and 2004. Two lines were created from the 2002 progeny, based on high breeding values (selection line) and for average breeding values (control line). The selection and control lines were then used as parents to produce progeny in the 2003 spawning season, and the progeny of the 2004 spawning season were produced by using the selection and control lines of 2003. In each spawning season, random samples of 100 progeny per family were tagged when their size reached 5 to 10g live weight. Two production environments were used to grow-out (for 120 days) the tagged progeny, that is ponds and cages. A number of statistical models were fitted to the data collected throughout the study, either to estimate breeding values (EBVs), variance components, or response to selection. Analysis was carried out using univariate model. The heritability estimated from the animal variance component was 0.386 (S.E. 0.0517) whereas the maternal and common environment effects estimated from the dam variance component was 0.139 (S.E 0.0201). Response to selection obtained was ranged from 6 to 13%.

Keywords: Selective breeding, *Oreochromis niloticus*, heritabilities, responses to selection

Abstrak: Kajian pembiakbakaan untuk berat badan berdasarkan pedigree penuh populasi tilapia strain GIFT (Genetically Improved Farmed Tilapia) telah dijalankan di Pusat Pengembangan Akuakultur, Jabatan Perikanan Malaysia, Jitra, Kedah. Progeni telah dihasilkan dalam tiga musim pembiakan iaitu 2002, 2003 dan 2004. Dua kumpulan telah dibentuk daripada progeni musim 2002, iaitu yang dipilih berdasarkan nilai pembiakan tertinggi (kumpulan terpilih) dan yang dipilih berdasarkan nilai purata pembiakan populasi (kumpulan kawalan) untuk berat badan. Kumpulan terpilih dan kawalan tersebut seterusnya digunakan sebagai induk untuk menghasilkan progeni dalam musim pembiakan 2003 dan progeni musim pembiakan 2004 dihasilkan menggunakan kumpulan terpilih dan kawalan daripada musim pembiakan 2003. Bagi setiap musim pembiakan, 100 sampel progeni diambil secara rawak dari setiap famili dan ditandakan (tag) apabila saiznya mencapai antara 5 hingga 10g seekor. Dua persekitaran pengeluaran digunakan untuk ternakan progeni yang telah ditanda tersebut iaitu kolam dan sangkar. Ternakan mengambil masa 120 hari. Beberapa model statistik telah digunakan ke atas data yang dikutip sepanjang kajian ini samada untuk menganggar nilai pembiakan (EBV), komponen varian atau tindakbalas pemilihan. Analisa telah dijalankan menggunakan model 'univariate'. Heritabiliti yang dianggarkan daripada komponen varian haiwan adalah 0.386 (S.E 0.0517) manakala kesan 'maternal' dan persekitaran yang dianggar daripada komponen varian induk betina adalah 0.139 (S.E 0.0201). Tindakbalas pemilihan yang dicapai adalah sekitar 6 hingga 13%.

Introduction

There are several ways for fish farmers to increase fish production either by augmenting the size of their fish farm or increasing productivity. Yields can be improved by environmental manipulations, such as the use of lime, fertilizers, feeds or improved water quality management. Growing genetically improved fish is another option and if both non-genetic and genetic approaches are jointly and wisely used, yields can increase dramatically. Selective breeding and crossbreeding are the two traditional approaches that have been used for thousands of years. Selective breeding is a technique that tries to improve the breeding value of the population by selecting and mating only the best fish (largest, heaviest, those with the desired colour and any other desirable attributes) in the hope that the selected brood fish will be able to transfer their superiority to their offspring. Selection is effective when a population has significant quantities of additive genetic variation, which is a significant heritability for the traits in question.

The main selection methods are mass or individual selection, family selection and combination of individual and family selection (or known as combined selection). All the methods focus on identification and evaluation of superior brood stocks to be used as parents of the future generation. In tilapia the focus of selection programs has been almost restricted to growth rate. Several estimates of heritability for live weight and growth rate of tilapia can be found in literature (Kronert *et al.*, 1989; Oldorf *et al.*, 1989; Gall and Bakar, 2002; Bolivar and Newkirk, 2002). One of the example of selective breeding program for tilapia is the GIFT project (Genetic Improvement of Farmed Tilapia) which was carried out in the Philippines between 1988 and 1997 by the WorldFish Center (formerly known as ICLARM), AKVAFORSK, Bureau of Fisheries and Aquatic Resources and the Freshwater Aquaculture Center of Central Luzon State University, Philippines. A combined family and within family selection was conducted to increase the growth rate of the Nile tilapia (*Oreochromis niloticus*). Selection was carried out once a year for five generations.

Since selective breeding is one of a tool to improve tilapia quality, this study was aimed at developing a plan for selective breeding in order to genetically improve tilapia quality for Malaysian aquaculture industry. Once a genetically improved breed is developed, it can be disseminated to fish farmers.

Materials and methods

Study location

The study was conducted at the Aquaculture Extension Centre, Department of Fisheries, Jitra, Kedah, Malaysia (latitude 6 N, longitude 100 E, altitude 23 m). The daily average temperature is 27 C, with little variation throughout the year. The annual rainfall is 2057 mm, occurring throughout the whole year but not in a uniform way. Rainfall in December, January and February (the driest months) is one half or less than in September and October (the wettest months).

Fish samples

The GIFT Foundation International Inc., Philippines, provided 63 full sib groups of 35 fish each, which were progeny from single pair mated parents (i.e. 63 males each mated to a different female). These fish belonged to the sixth generation of selection of GIFT strain, and were used as the base population for this genetic improvement project. No selection took place among the fish transferred from the GIFT Foundation, since they were received in batches and there were uncertainties regarding environmental factors that could be influencing their performance.

The fish were reared until an average body weight of about 250g before mating was initiated. They were mated and produced the 7th generation in the spawning season of 2002, which in turn produced the 8th and 9th generations in 2003 and 2004 respectively. Two lines were created with the 2002 progeny, one selected based on high breeding value for live weight (selection line), and another one selected for average

breeding values (control line). The data structure from the generation produced in 2002 to the generation produced in 2004 is shown in Table 1. This data structure is relevant for univariate analysis where harvest weight is treated as a single trait. Figure 1 shows a schematic diagram summary of production for generation G1, G2 and G3 in spawning seasons 2002, 2003 and 2004 respectively.

Breeding in hapas

Breeding was conducted in hapas installed in ponds. A hapa is a fixed net enclosure, similar to an inverted mosquito net, made of polyethylene netting with joints in nylon thread. The standard size of hapa used for breeding was 1m (width) x 1m (length) x 1m (depth) with 2 mm mesh size. The breeding hapas enabled a controlled production and maintenance of large numbers of full and half-sib families.

Mating design

The production of families was conducted in 1m x 1m x 1m breeding hapas according to the mating plan prepared based on the estimated breeding value of the selected breeders (ASReml software). Generally, each male breeder was mated to two or three female breeders (in separate hapas) to produce paternal half-sib families. This enabled the calculation of phenotypic and genetic parameters. Mating of closely related individuals was avoided to prevent inbreeding depression.

Table 1: Number of sires, dams and progeny, by spawning season and line

Spawning Season	Line	Sires	Dams	Progeny
2002 (G1)	Base Population	52	54	1684
2003 (G2)	Selection	35	65	2560
	Control	19	19	1150
2004 (G3)	Selection	54	84	3714
	Control	17	22	957
Total		177	244	10065

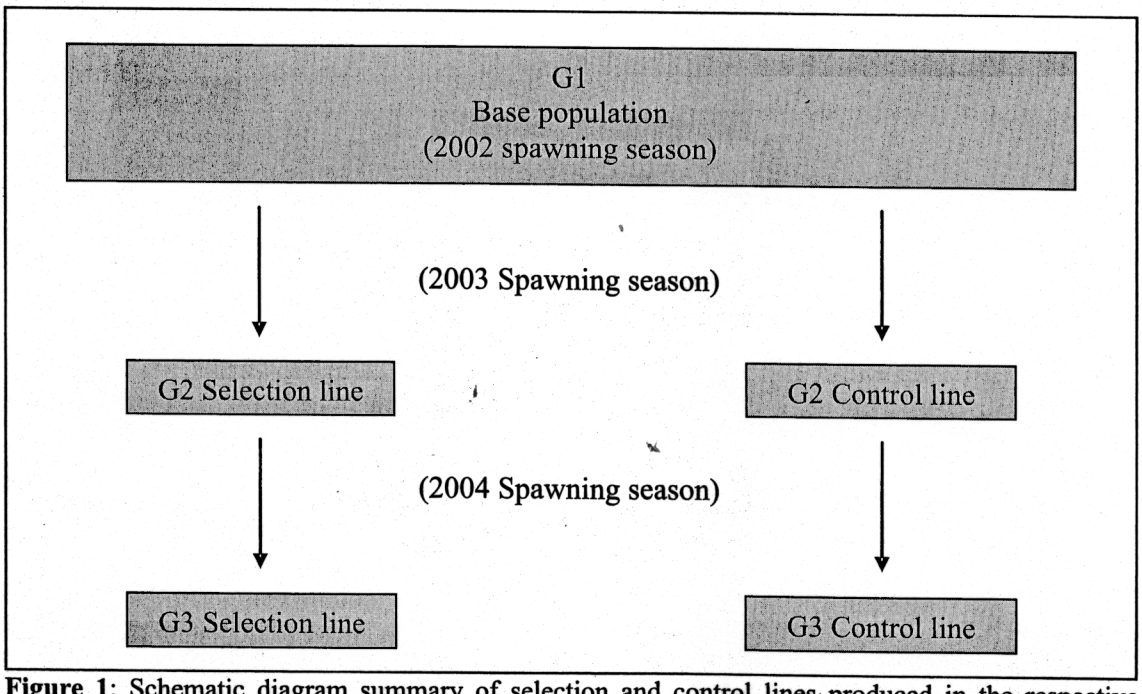

Figure 1: Schematic diagram summary of selection and control lines produced in the respective spawning seasons of 2002, 2003 and 2004

Breeding procedure

Before mating, the males and females were conditioned in separate tanks for a week. A total of 140 breeding hapas was used in each mating cycle. The female breeders were stocked into the breeding hapas before the males. Only the most 'ready to spawn' females were paired with the male in the hapa. After a week of mating, fertilized eggs were collected from the mouth of the female and immediately transferred to hatching jars. The date of spawning was recorded for each individual pair mated. The male was then paired to the second female in another hapa. The male and female breeders were mated again if they produced less than 200 fry. The breeders were not fed when the females were expected to spawn in order to prevent them from swallowing their eggs.

Hatching jar

Eggs that were collected from the female breeder's mouth were transferred to hatching jars made of fibreglass. The design and system of the jars acted as an artificial incubator for the fertilized eggs. There was a constant flow through of filtered water to optimize the environment for the eggs. Eggs from each female were stocked in the respective jar for three to five days until hatching. The hapa number was recorded on the jar as an identification of family. To ensure a good hatching rate, the water temperature was maintained in the range of 26°C to 30°C.

Rearing of fry

The hatched fry from the incubators were transferred into nursery hapas (1m x 1m x 1m with 2mm mesh size) according to their parents or family number at a density of 200fry/m^3. The total live weight and quantity of fry were recorded before transferring them into hapas. At least three replicates of nursery hapa for each family were maintained in the same pond to reduce environmental differences between families. They were reared for 21 days in nursery hapas and then transferred to bigger mesh size (8mm) hapas (1m x 1m x 1m) called B-net cages. The stocking density in the B-net cages was reduced to 120fry/m^3. The purpose of using B-net cages was to allow better water circulation. Rearing in the B-net cages took another 21 days until the fry live weight reached 5 to 10g and were ready to be tagged.

Breeding data

Data were collected during the conduct of the breeding activity, beginning with the mating of breeders, egg collection, nursing of fry and tagging. Live weight of all breeders was recorded before mating. Recording was also done on the number of eggs per female breeder, number, total live weight and date of hatching, number of fry per nursery hapa and number of fry transferred and collected from B-net cages. These records were important to ensure that enough fry were produced and that the rearing conditions were standardized for all the families.

Fish identification (tagging)

Fish identification is one of the most important tasks to be conducted in a selective breeding program. Accurate testing of the fish in farm environments requires individual or group identification. Note that as earlier described, pair matings were conducted and the identity of full and half-sibs was retained until tagging time, which enabled the maintenance of a fully pedigreed population.

In this study, three types of tagging were used for different generations: PIT (Passive Integrated Transponder) tag, floy tag and T-bar anchor tag. Fingerlings were anesthetized before tagging using MS 222 solution (1ppt). The base population was identified using PIT tag. Twenty individuals per family were tagged before the culture trials. Random sample from the first and second generations were identified using floy tag (100 individuals per family). However the third generation was marked with floy tag (100 individuals per family) and T-bar anchor tag (20 individuals per family) for culture trials. In all generations, the tag number, live weight (LW), standard length (L), body depth (D), body width (W) and sex were recorded before stocking.

The test environments

After tagging, the fish were grown either in cages or in earthen ponds. The cages were located in an irrigation canal at Kodiang, Kedah, 22 km away from Jitra. Eight 3m long by 3m wide cages adjacent to each other were established, and the fish were assigned at random to them. The initial stocking density was 55 fish per m^2 of surface water. The fish were fed at 3 to 5 per cent of their live weight. A commercial dry pelleted feed with 32% crude protein content was given twice a day (i.e. at 8.30a.m and 5.00p.m.). Water quality parameters (temperature, pH, dissolved oxygen) were monitored once a week.

The 0.1ha earthen ponds located at the Aquaculture Extension Centre, Department of Fisheries, Jitra, Kedah. The initial density in each pond was 3fish/m^2 of surface water. The same quantity of feed and feeding frequency was used as for the cages. Water quality parameters were also monitored once a week.

Harvesting

At the end of the grow-out period, all the test fish were harvested. In the case of cages fish were harvested by lifting up the net, and putting them into aerated tanks by using a scoop net. They were later conditioned in tanks at the Jitra Station. A seine net was used in the ponds to harvest the fish. The ponds were completely dried in the following day, early in the morning to minimize stress. The fish were then transferred to conditioning cages (3m x 3m x 1m) installed in another pond.

Data collections

Data recording of all the harvested fish was done three days after conditioning them in tanks or cages. The individual tag number, sex, individual live weight (LW), standard length (L), body width (W) and body depth (D) were recorded. Width and depth were measured at the mid-side of the fish, where they were greatest. Sex of the fish was also recorded as well as visual assessment of female sexual maturity. After recording, the breeders were transferred back to their respective conditioning cages until data analysis was completed. The age (in days) of each individual fish was computed based on the harvesting and hatching dates.

Data analysis

The data were first examined using SAS (1990) application software to calculate simple statistics, remove anomalies and conduct a preliminary selection of the statistical models to be fitted. The procedure PROC MIXED (SAS Institute Inc., 1997) was used to estimate the fixed effects and initial values of variance components, in which case sire (nested within spawning season and line) and dam (nested within sire, spawning season and line) were fitted as random effects. Non-significant two way interactions among the fixed effects were deleted from the model. In a second phase, the computer program ASReml was used (Gilmour *et al.* 2002). The models fitted included the fixed effects of spawning season (2002, 2003 and 2004), selection line (Selection and Control), environment (cage and pond), sex, and two-way interactions among them.

Animal and dam (the non-genetic component) were fitted as random effects, whereas age of the fish was used as a covariate. The sub-set of these effects that was fitted for different purposes is shown and discussed in the Results section. Non-significant two-way interactions among the fixed effects were deleted from the model. On further examination, it was noted that the remaining interactions between fixed effects were unimportant and never involved reversal of rankings for levels of one effect in levels of another. For that reason, and because they negligibly contributed to the goodness of fit of the model, all two-way interactions among fixed effects were finally discarded.

The analysis enabled the estimation of (animal model) breeding values for all fish, and these were used in making selection decisions in the selection and control lines, and in estimating the genetic trend. They also enabled the estimation of variance components, from which phenotypic and genetic parameters were calculated.

For variance component estimation with ASReml, spawning season, line, sex, and environment classes were fitted as it was the model resulting in the greatest log likelihood value. Age at harvest was used as a covariate. The availability of a complete pedigree in the population enabled fitting an animal (random) model. Dam was fitted as another random effect, but solely accounting for the environmental effect on the progeny, without a genetic structure. The dam variance component (σ^2_D) was in this case a combination of the maternal effect and the common environment (so $\sigma^2_D = \sigma^2_{M\_Ec}$) to which full sibs were exposed early in life (that is, while being hatched and while grown in the nursing and rearing hapas).

The animal variance component provided the estimate of the additive genetic variance (σ^2_A), whereas the phenotypic variance (σ^2_P) was estimated from the sum of all variance components. The heritability (h^2) was computed as the ratio between the additive genetic and the phenotypic variances. The maternal and common environmental effect (c^2) was calculated as the ratio between the dam variance component and the phenotypic variance ($c^2 = \sigma^2_D / \sigma^2_P$ or $\sigma^2_{M\_Ec} / \sigma^2_P$)

Selection method

The progeny resulting from the 2002 spawning season were selected as parents of the next generation (G2) in two different ways, to create the Selection line and to continue the Base population as the Control line. The parents for the Selection line were selected from among those with the greatest breeding values whereas the parents of the Control line were selected among those with breeding values as close as possible to the average. In order to ensure that the breeding value could be estimated properly, inbreeding was restricted by avoiding mating of full sibs, half sibs or cousins and maintaining the effective population. The same procedure was followed to produce the next generation in 2004 spawning season (G3).

Results and Discussion

Descriptive Statistics

Table 2 shows the simple statistics obtained by using Proc Means in SAS. The coefficient of variation for LW was very high in all the three generations. Furthermore, when inspecting the residuals from the analysis of variance it was found the greater means were associated with greater variances in the sub-classes. To improve the distribution, LW was square root transformed in all the later analyses.

Estimates of fixed effects

Table 3 indicates that all the fixed effect and their interaction subclasses were statistically significant ($p<0.05$) except for environmental effect.

Phenotypic and genetic parameters

Results in Table 4 were generated by using ASReml software. There was an additive genetic variance present (2.7258). The heritability was high and there was maternal common environmental effect of low magnitude present (0.139). The estimated heritability (0.386) was within acceptable magnitude as reported by previous study (Kronert *et al.*, 1989).

Table 2: Number of observations (N), simple mean, minimum and maximum, standard deviation and coefficient of variation and standard deviation of LW (g) and age (days) at harvesting

Variable	Spawning Season	N	Mean	Minimum	Maximum	Standard Deviation	Coefficient of variation (%)
Live Weight (LW)	2002	1684	227.1	29.0	608.0	101.5	45
	2003	3710	154.1	7.0	617.0	86.3	56
	2004	4671	192.8	19.0	682.0	101.4	53
Age at Harvesting	2002	1684	256.7	215.0	280.0	12.8	5
	2003	3710	213.9	125.0	265.0	25.3	12
	2004	4671	244.6	173.0	302.0	28.8	12

Selection response

The response to selection was obtained by comparing the estimated breeding values between generations and between lines. The results showed that genetic change occurred in this study (Table 5). The values of the response are presented in three different ways namely, in actual units, as a percentage of the mean and in genetic standard deviation units. All the results indicated high response to selection. This suggested that genetic change was being achieved.

Table 3: Analysis of variance for $LW^{0.5}$: Tests of fixed effects using PROC MIXED

Effects	F Value	Prob. > F
Spawning Season (SS)	36.84	< 0.0001
Line (L)	22.78	< 0.0001
Sex (S)	67.12	< 0.0001
Environment (E)	1.37	0.2426
Age (SS, S, E)	371.19	< 0.0001
Residual Variance	4.259	

Table 4: Variance components, heritability and maternal common environment effect for $LW^{0.5}$

Parameter	REML Estimate
Additive genetic variance (σ^2_A)	2.7258
Maternal and common environment variance ($\sigma^2_D = \sigma_{M\_Ec}{}^2$)	0.9809
Phenotypic variance (σ^2_P)	7.0548
Heritability (standard error) [h^2 (S.E.)]	0.386 (0.0517)
Maternal common environment (standard error) [c^2 (S.E.)]	0.139 (0.0201)

Table 5: Response to selection estimated by different methods and expressed in different ways

Selection Response ($LW^{0.5}$)	Actual units[A] ($g^{0.5}$)	Percentage	Genetic Standard Deviation Units (Actual/σ_A)
Between 2002 spawning season and 2003 spawning season.	0.954	7.25	0.578
Between 2003 spawning season and 2004 spawning season.	0.796	6.05	0.482
Between control and selected line in 2003 spawning season	1.133	8.61	0.686
Between control and selected line in 2004 spawning season	1.732	13.16	1.049

Note: [A] Actual units are $LW^{0.5}$ difference in mean breeding values; percentage refers to actual units, in relation to the least squares means of $LW^{0.5}$ for the control population ($13.1563 g^{0.5}$); Genetic standard deviation equals the square root of the additive genetic variance in Table 4 ($_A = 1.651 g^{0.5}$)

Conclusion

The present study indicated that genetic enhancement of growth rate had been achieved through selective breeding. The estimated genetic gains from the three generations of selection ranged from 6 to 13 percent and it is in good agreement with similar studies reported by other researchers.

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References

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

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

Eknath, A.E. and Acosta, B.O. 1998. Genetic Improvement of Farmed Tilapias (GIFT) project: Final report (March 1988 to December 1997). International Center for Living Aquatic Resources Management, Makati City, Philippines.

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

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J. and Thompson, R. 2002. ASReml user guide (Release 1.0). VSN International Ltd. Hemel Hempstead, HP 1 1ES, UK.

Gjedrem, T. 1999. Aquaculture needs genetically improved animals. The Advocate (December 1999). 69-71 pp.

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

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

SAS Institute Inc., 1990. SAS/STAT^{R} User's guide (Version 6, Fourth Edition, Vols. 1 and 2). Cary, NC, USA.