Genetic Analysis of the GIFT Strain (Nile Tilapia, *Oreochromis niloticus*) in Malaysia

 $H.L.\ Khaw^{*\dagger}$, R.W. Ponzoni * , A. Hamzah ‡ , N.H. Nguyen * , K.R. Abu-Bakar * , H.Y. Yee *

Introduction

Tilapia is an African fish, famous for its hardiness, good market value and affordable price (Shelton 2002). The majority of tilapia culture is outside Africa (Shelton 2002). Asia is the largest producer of farmed tilapia (FAO 2009). Several tilapia selective breeding programs have been established in Asian countries (Bolivar 1998; Gupta and Acosta 2004; Tayamen 2004; Zimmermann and Natividad, 2004).

The Genetically Improved Farmed Tilapia (GIFT) strain is well known worldwide because of its high performance. A first phase of the project ended in 1997 after five generations of selection (Gupta and Acosta 2004). In 2002, the GIFT population in the WorldFish Center Malaysia was established based on the sixth generation of GIFT from Philippines (Ponzoni *et al.* 2005). In Malaysia, the breeding program continued the selection for live weight at harvest time (LW) to improve the growth rate. The GIFT population has already undergone seven generations of selection since it was introduced in Malaysia. A review of its progress and performance is timely in order to decide the program's future direction. In this paper we report phenotypic and genetic parameters for live weight based on the data collected over eight generations, as well as the selection response achieved during that period.

Material and methods

The fish and the environment. The selective breeding program for GIFT in Malaysia was conducted at the Aquaculture Extension Center located at Jitra in Kedah State of Malaysia. Between the end of 2000 and the beginning of 2001, the GIFT Foundation in Philippines transferred 2205 fish (in batches) from 63 full-sib families to the WorldFish Center in Malaysia. In the spawning season of 2002 (SS02), we produced the base population of GIFT in Malaysia by mating the fish we received. With the 2002 progeny, two lines were created: the Selection line (S), selected for high breeding value for live weight, and the Control line (C), selected for average breeding value. In the S line, one male was mated to two females, whereas one male was mated to one female in the C line. At the spawning season of 2009 (SS09), GIFT had undergone seven generations of selection. The generation interval is one year and the generations are discrete. Details on experimental design for the breeding program can be found in Ponzoni *et al.* (2005, 2010).

^{*}The WorldFish Center, Jalan Batu Maung, 11960 Bayan Lepas, Penang, Malaysia.

[†] Animal Breeding and Genomics Centre, Marijkeweg 40, 6709 PG Wageningen, The Netherlands.

^{*} National Prawn Fry Production and Research Center, Kampung Pulau Sayak, 08500 Kedah, Malaysia.

Records and statistical analysis. Fish were individually tagged with PIT (Passive Integrated Transponder) tags in order to maintain the full pedigree information. Live weight, standard length, width and depth were measured at harvest time. Each fish was visually sexed. The age of each fish (in days) was calculated from the spawning and harvesting dates. The data collected over eight generations consisted of records from 27,502 progeny produced by 473 sires and 699 dams. During the preliminary analysis, PROC MIXED (SAS Institute Inc., 1997) was used to estimate the fixed effects (spawning season: SS02 to SS09, line: SEL and CON, environment: cage and pond, and sex) and their two way interactions. Sire and dam (nested within sire) were fitted as random effects.

Variance components were estimated using restricted maximum likelihood (REML) methods, implemented by the ASReml computer program (Gilmour et al. 2002). In the preliminary analysis, the interactions among fixed effects were either statistically non-significant or deemed unimportant (due to scale). For variance component estimation, we fitted "spawning season, line, environment, sex" sub-classes. Age of the fish was fitted as covariate with the spline option in ASReml (Gilmour et al. 2002). The availability of the full pedigree information enables fitting a random animal effect (estimating σ^2_A) and a random dam effect (σ^2_D , solely accounting for maternal and common environmental effects on progeny, without genetic structure).

The heritability (h²) and common environmental effect (c²) were calculated as: $h^2 = \sigma^2_A/(\sigma^2_A + \sigma^2_D + \sigma^2_e)$, and $c^2 = \sigma^2_D/(\sigma^2_A + \sigma^2_D + \sigma^2_e)$, respectively. The square root transformation of live weight improved the distribution of residuals and was used in all the analyses. The selection response in GIFT was estimated in two ways: (i) comparing the estimated breeding value (EBV) for live weight of progeny from the S line between generations, and (ii) comparing the EBV of progeny between the two lines (S and C) in each generation. In this paper we only present the total selection response to the latest (eighth) generation.

Results and discussion

Table 1 shows descriptive statistics for live weight and age at harvest over eight generations in the GIFT population in Malaysia. The coefficient of variation for live weight was strikingly high. The average survival rate to harvest time was about 80%.

Table 1: Number of observations (n), simple mean (μ) , standard deviation (SD) and coefficient of variation (CV) of LW and age at harvesting.

	()		8-	
Variable	n	μ	SD	CV (%)
LW	27502	211	91.2	43
Age at harvesting	21302	238	28.7	12

Table 2 shows the analysis of variance for live weight. All the main effects and the covariate were highly significant with p<0.0001, except for the environment effect that had a significance level of p<0.05. This could be due to the fact that since the fourth generation we

only grow-out the fish in earthen ponds, having discontinued the cage environment. We estimated the genetic correlation for live weight between cages and ponds environment, 0.75±0.09, which indicated moderate to low genotype by environmental interaction (Khaw *et al.* 2009).

Table 2: Analysis of variance for LW^{0.5}: Tests of fixed effects using PROC MIXED.

F Value	Prob. > F
35.70	< 0.0001
86.44	< 0.0001
139.77	< 0.0001
5.07	0.0244
329.18	< 0.0001
2.8	8508
	35.70 86.44 139.77 5.07 329.18

Table 3 shows the REML estimates of phenotypic and genetic parameters. The results show the presence of additive genetic variance, resulting in a moderate heritability, 0.30 ± 0.035 . Our heritability estimate was in good agreement with other estimates, published in the literature (0.34, Ponzoni *et al.* 2005; 0.26, Rutten *et al.* 2005; 0.32, Maluwa *et al.* 2006). The estimate indicates that the additive genetic variance shows no sign of diminishing due to selection over time. The estimated c^2 for live weight is greater than other estimates in literature (0.15, Ponzoni et al. 2005; 0.21, Rutten et al. 2005; 0.09, Maluwa et al., 2006). The large c^2 is due to keeping the full sibs together until they reach the tagging size of 10g (average age is 90 days) in order to obtain the full pedigree information. The c^2 can be reduced by having a more uniform nursing environment for the fry before they reach the tagging size (Maluwa *et al.* 2006) or by genotyping the fingerlings to ascertain parentage. For a developing country, the latter method is still too expensive to implement. Although the c^2 can be reduced by better management or techniques, the maternal effects could still be there via egg size and initial mouth brooding for Nile tilapia (Khaw *et al.* 2009)

Table 3: Variance components, heritability and maternal common environment effect for LW^{0.5}.

IOI EV		
Parameter	REML Estimate	
Additive genetic variance (σ^2_A)	1.81	
Maternal and common environmental variance (σ^2_D)	2.05	
Phenotypic variance (σ^2_P)	6.03	
Heritability (standard error) [h ² (s.e.)]	0.30 (0.035)	
Maternal common environment (standard error) $[c^2 (s.e.)]$	0.34 (0.018)	

In this study, both methods used in estimating the genetic change were in good agreement with each other. Comparing the average EBV of the base population with the latest generation (eighth generation), the estimated genetic gain was 52.5%, whereas comparing the average EBV of the C and S lines at the eighth generation, the estimated genetic gain was 52.2%. Note that our % estimates are in square root units and that in actual units those percentages would be double (i.e. ~ 100%) (James 2007). According to Gjedrem (2000), the estimates of genetic gain per generation for aquatic animals range from 10% to 20%. In our case, the estimates fall within Gjedrem's prediction. They are also in agreement with those

reported by Gall and Bakar (2002). Based on our estimates using Method (i) (results not presented in this study), we can see that the genetic gain has declined since the sixth generation (from 9% drop to 5%). The decline of genetic gain could due to the high losses during the last three generations (caused by natural calamity) and directly caused a lower selection intensity and loss of valuable fish. Note also that selection intensity in any generation is lower that the potential because of the management of inbreeding and effective population size in this population. This entails a trade-off between immediate and long-term gains (Ponzoni et al. 2010).

Conclusion

GIFT is an improved strain with proven high performance (Dey et al. 2000). Since its introduction to Malaysia it has been disseminated to nine countries in Asia and Latin America. The presence of additive genetic variance in harvest weight augurs well for future selection response in that trait. The mate allocation strategy (Ponzoni et al., 2010) has contained inbreeding and maintained effective population size. The program is well positioned to consider the incorporation of other traits in the breeding objective (e.g. flesh quality, disease resistance, behavioural traits), especially if population size were increased, perhaps by linking the Malaysian GIFT with well managed nuclei in other countries. We face a major challenge in further developing this outstanding strain.

Acknowledgement

Part of this research was financially supported by the Dutch science council (NWO-STW). The GIFT project in Malaysia is funded by European Union and Department of Fisheries Malaysia.

References

Bolivar, R.B. (1998). PhD. Dissertation, Dalhousie University, Canada, 166pp.

Dey, M.M., Eknath, A.E., Li, S., et al. (2000). Aquaculture Econ. Manage., 4: 83-106.

Gupta, M.V. and Acosta, B.O. (2004). NAGA, 27 (3 and 4): 4-14.

FAO (2009). «FAO yearbook. Fishery and Aquaculture Statistics», Rome. 72pp.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., *et al.* (2002). «ASReml User Guide Release 1.0», VSN International Ltd, Hemel Hempstead. HP1 1ES, UK.

Gjedrem, T. (2000). Aquaculture Research, 31: 25-33.

James, J.W. (2007). *Proc.* 17th AAABG, p. 150-153.

Khaw, H.L., Bovenhuis, H., Ponzoni, R.W., et al. (2009). Aquaculture, 294: 37-42.

Khaw, H.L., Ponzoni, R.W., Hamzah, A., et al. (2009). Proc 18th AAABG, p. 60-63.

Maluwa, A.O., Gjerde, B., Ponzoni, R.W. (2006). Aquaculture, 259: 47-55.

Ponzoni, R.W., Hamzah, A., Tan, S., et al. (2005). Aquaculture, 247: 203-210.

Ponzoni, R.W., Khaw, H.L., Nguyen, N.H., et al. (2010). Aquaculture, In Press.

Rutten, M.J.M., Komen, H., Bovenhuis, H. (2005). Aquaculture, 246: 101-113.

SAS Institute Inc. (1997). «Changes and Enhancements». NC, USA. 1167pp.

Shelton, W.L. (2002). Proc Int. Forum on Tilapia Farming in the 21st Century, p. 1–20.

Tayamen, M.M. (2004). *Proc* 6th *ISTA*, p. 74-88.

Zimmermann, S. and Natividad, J.M. (2004). Proc 6th ISTA, p. 89.