SELECTION FOR LIVE WEIGHT IN THE GIFT STRAIN OF NILE TILAPIA (*Oreochromis niloticus*)

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Abstract

A fully pedigreed population based on the sixth generation of GIFT (Genetically Improved Farmed Tilapia) was established in Malaysia in 2002. Progeny were generated in two spawning seasons, 2002 and 2003. 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. Parents used in the spawning season of 2003 were either selected as having high estimated breeding values for live weight (LW) at approx. 7 months of age, or as having EBVs as close as possible to the average. In this way a Selection and a Control line were created, respectively. Two production environments were used to grow-out the progeny. At approx. 7 months of age females' live weight was 84 per cent that of males, whereas live weight in cages was 83 per cent of that in ponds. The heritability estimated from the animal variance component was 0.31 (s.e. 0.069), whereas the maternal and common environment effect estimated from the dam variance component was 0.15 (s.e. 0.031). Response to selection was estimated by three methods. Expressed as a percentage of the overall least squares mean for LW in the population, the response was about 10 per cent. The results are discussed in relation to other work. It was concluded that there was still additive genetic variance in the GIFT population established in Malaysia, and that it was capable of further response to selection. The issue of genotype by environment interaction is briefly discussed, and it was concluded that there was no justification for the conduct of separate genetic improvement programs in cage and in pond environments.

Introduction

In Tilapia the focus of selection programs has been almost exclusively restricted to growth rate. Several estimates of heritability, in particular for live weight and growth rate, can be found in the literature (e.g. Kronert *et al*. 1989, Oldorf *et al*. 1989, Gall and Bakar 2002, Bolivar and Newkirk 2002). In a strict sense, such genetic parameters are only applicable to the population and the environment where they were obtained. Furthermore, individual estimates are subject to sampling problems and the parameters can change over time. Hence, the desirability of having parameter estimates that are directly relevant to the population one is working with. In this paper we present estimates of heritability for live

weight (at approx. seven months of age) for fish of the GIFT (Genetically Improved Farmed Tilapia) strain (Eknath *et al*. 1993, Bentsen *et al*. 1998, Eknath and Acosta 1998), grown out in two environments (cage and pond). We also estimate the response to selection in harvest weight by three different methods. The issue of possible genotype (individual's genetic merit) by grow-out environment (cages or ponds) interaction is briefly examined. The results are presented in greater detail in two papers submitted for publication (Ponzoni *et al*. 2004a, b).

Materials and methods

The environment

The work was conducted at the Aquaculture Extension Center, Department of Fisheries, Jitra, Kedah State, 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 during 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).

The fish

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 actual selection of GIFT (without counting the generations over which the composite base population was created), and were received at Jitra in batches towards the end of 2000 and during the beginning of 2001. They were mated and produced a seventh generation in the spawning season of 2002, which in turn produced an eighth generation in 2003. 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. Two lines were created with the 2002 progeny, one selected on high breeding value for live weight (Selection line, S), and another one selected for average breeding values (Control line, C). The number of sires and dams from which progeny was harvested in both spawning seasons and lines, as well as the number of progeny, are shown in Table 1. The numbers were less than planned, mainly due to tag losses, but also partly due to mortality and elimination from the final data set of some individuals considered outliers. None of the parents used in the 2002 spawning season were used in 2003 (i.e. generations were discrete). Note that we consider the progeny produced in the 2002 spawning season our Base Population, and in our analyses we treat it as part of the established Control line.

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

Spawning season	Line	Sires	Dams	Progeny
2002	Base population	52	54	1684
	Selection	35	65	2560
2003	Control	19	19	1150
Total		106	138	5394

The reproduction and management schedules for 2002 and 2003 are shown in Table 2. The methodology used is described in the publication WorldFish Center (2004). Following the grow-out period the fish were harvested in the dates indicated in Table 2.

Table 2. Schedule of reproduction and management.

Activities	Spawning season	
	2002	2003
Mating	February and March	January and February
Nursing hapas	February to April	January to March
Rearing hapas	March to May	February to April
Tagging	April to May	March to April
Grow-out	Ponds: June to October Cages: July to November [A]	Ponds: April to August Cages: April to September
Harvest	Ponds: 28 to 31 October Cages: 9 to 13 November	Ponds: 18 to 25 August Cages: 2 to 17 September

[A]Delayed stocking in cages because of the small size of fry in June

The grow-out system

After tagging the fish were grown out either in cages or in earthen ponds. The cages were located in flowing water in an irrigation canal at Kodiang, Kedah, 22 km away from Jitra. Four 3m long by 3m wide by 2.1m deep 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 an amount equivalent to 3 to 5 per cent of their live weight per day. A commercial dry pelleted feed with 32 per cent protein content was fed twice a day (i.e. at 8.30 a.m and 5.00 p.m.). The 0.1 ha earthen pond was 0.1 located at the Aquaculture Extension Center, Jitra. The initial density in the pond was three to four fish per m^2 of surface water. The same feeding rate and frequency was used as for the cages. All the fish were harvested after (approx.) 120 days of grow out in either (cages or pond) of the environments.

Records

Data recording of all the tagged fish was done at harvest, when individual live weight (LW), total length, width and depth were measured. Width and depth were measured at the mid-side of the fish, where they were greatest. Sex of the fish was also recorded, and a subjective visual assessment was made of female sexual activity using the categorization

described in WorldFish Center (2004). From the harvesting and spawning dates we are able to compute the age (in days) of each individual fish. In this paper we only report on the results for LW. Those for the other traits will be reported elsewhere.

Data analysis

The data were first examined using SAS (1990) to calculate simple statistics, remove anomalies and conduct a preliminary selection of the statistical models to be fitted. 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 and 2003), selection line (S and C), 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 varied, depending on the purpose of the particular analysis. Non-significant two-way interactions among the fixed effects were deleted from the model. On further examination we 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 one. 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 analyses 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.

Results

Descriptive statistics

Table 3 shows (for both seasons combined) the number of observations, simple mean, minimum and maximum, standard deviation and coefficient of variation values for LW in the two environments, and for age at harvest. The coefficient of variation was strikingly high. Plotting of residuals during preliminary analyses indicated that greater means were associated with greater variances. The square root transformation of LW improved the distribution of residuals and was used in all later analyses. Over the two spawning seasons the range in age at harvest was greater than within any one of the seasons due to harvesting at an earlier age in 2003 relative to 2002 (see Table 2).

Table 3. Number of observations (N), simple mean, minimum and maximum, standard
deviation and coefficient of variation of LW (g) and age (days) at harvesting.

Variable	N	Mean	Minimum	Maximum	Standard Deviation	Coefficient Variation (%)
LW (Cages)	3197	166	13	591	80.4	48
LW (Pond)	2197	192	7	617	116.1	60
Age at harvesting	5394	227	125	280	29.7	13

Estimates of sex and environmental effects

In the preliminary analysis the fixed effects of spawning season, line, sex and
environment were fitted as fixed effects using PROC MIXED (SAS 1997), as well as all
possible two-way interactions. The latter were deleted from the model as earlier stated. Age
of the fish was fitted as a linear covariate within spawning season, sex and environment. Sire
(nested within spawning season and line) and dam (nested within sire, spawning season and
line) were fitted as random effects. All the earlier mentioned fixed effects and the covariate
were statistically significant ($P < 0.0001$). Table 4 shows the least squares means for LW in
females and males, for cages and ponds. The differences between the sexes and between the
environments were statistically significant. They were consistent with other reports in the
case of sex, and with our observations in relation to growth rates in cages and pond. In both
environments, females' LW was 84 per cent that of males, whereas, averaged over both
sexes, LW in cages was 83 per cent of that in ponds.

Table 4. Live weight least squares means for environment by sex combinations.

Environment	Sex	Least Squares Means (g)
Cages	Female	191_a (8.2)[A]
	Male	223_b (8.1)
Pond	Female	228_b (6.4)
	Male	272_c (6.3)

[A]Analysis conducted on LW, the significance levels were the same as for $LW^{0.5}$. Means with
the same subscript do not differ significantly from each other. Standard errors in brackets.

Phenotypic and genetic parameters estimated with ASReml

Because in the preliminary analyses interactions were either statistically non-significant or deemed unimportant (due to scale and not to reversal of rankings), for variance component estimation we fitted 'spawning season, line, sex, environment' classes (altogether 12 combinations). Age at harvest was used as a covariate, with the 'spline' option available in ASReml. 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) is 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 are exposed early in life (i.e. while being hatched and while 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 in the usual way, 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. The REML estimates of variance components, heritability and maternal common environmental effect are shown in Table 5.

Table 5. Variance components, heritability and maternal common environment effect for $LW^{0.5}$.

Parameter	REML Estimate
Additive genetic variance (σ^2_A)	2.6821
Maternal and common environment variance ($\sigma^2_D = \sigma_{M\_Ec}^2$)	1.2012
Phenotypic variance (σ^2_P)	7.9559
Heritability (standard error) [h^2 (s.e.)]	0.34 (0.069)
Maternal common environment (standard error) [c^2 (s.e.)]	0.15 (0.031)

Estimation of response to selection from ASReml analyses

The progeny resulting from the 2002 spawning season were selected as parents of the next generation in two different ways, to create the Selection line, and to continue the Base Population as the Control line. Animal model breeding values were calculated for all individuals fitting a statistical model like the one described above, except that it did not include spawning season. The parents for the Selection line were selected from among those with the greatest breeding values for LW, imposing some restrictions with the aim of controlling inbreeding and maintaining a high effective population size. By contrast, the parents of the Control line were selected among those with breeding values for LW as close to the average as possible, and imposing the same sort of restrictions regarding inbreeding and population size as for the Selection line. We estimated the genetic change in LW in three ways: (i) Comparing the least squares means for the Selection and Control lines in the progeny of the 2003 spawning season; (ii) Comparing the estimated breeding values for LW between the progeny of the 2002 spawning season and those of the Selected line in the 2003 spawning season, and (iii) Comparing the estimated breeding values of the Selection and Control lines in progeny of the 2003 spawning season. The results are shown in Table 6 in

which the model fitted in each case is also specified. Overall, there was good agreement among the methods, although the estimate from method (iii) was greater than for the other two methods. In all cases the response was large enough to suggest that genetic change was being achieved, and in the intended direction.

Table 6.   Response to selection in LW estimated by different methods.

Method	Model (effects)	Selection Response ($LW^{0.5}$) Percentage[A]
(i) Difference between the least squares means for LW for the Selection and the Control lines in the progeny of the 2003 spawning season	Fixed: SS, L, S, E, SSxS, LxS Covariate: age (SS, S, E)	8.4
(ii) Difference between the estimated breeding values for LW in the progeny of the 2002 spawning season and the estimated breeding values of the Selected line in the 2003 spawning season	Fixed: SSxSxE Covariate: Age (SS, S, E) Random: animal, dam	8.7
(iii) Difference between the estimated breeding values for LW of the Selection and the Control lines in the progeny of the 2003 spawning season.	Fixed: SSxSxE Covariate: Age (SS, S, E) Random: animal, dam	11.4

[A]Percentage refers to actual units, in relation to the least squares mean of $LW^{0.5}$ for the whole population (14.7 $g^{0.5}$); actual units are $LW^{0.5}$ difference in mean values for method (i) and difference in mean breeding values for methods (ii) and (iii).

Discussion

General

The results reported in this paper are part of a long term project on the further genetic improvement of GIFT, and they represent an early stage in the development of improved Nile Tilapia. As such, they should not be viewed as definitive, but only as indicative of features that appear to begin emerging.

Environmental effects

We observed large variability in LW for both environments, cages and ponds. Note however, that this variability was within the range reported in the literature for other aquatic

species (Gjedrem 2000, Hallerman 2003), but it was greater than that reported for terrestrial domestic livestock (Simm 1998, pp. 46-50).

Males were always heavier than females by about 16 per cent (Table 5). In ponds there was some evidence of female reproduction at harvest time. This finding is consistent with other reports (Lorenzen 2000) and lends support to the interest displayed by many researchers in the production of 'all male Tilapia' for grow-out operations (see review by Penman and McAndrew 2000).

The greater weight in ponds than in cages is most likely, largely a reflection of the density of the fish in both environments and of the availability of natural food. In either case the density was chosen after surveying producers' practices in the region where the research station is located. The results suggest that at such densities, and with the feeding regime and management adopted, the cage production system has the advantage of housing a large number of fish in a small area, but growth rates are likely to be lower than in ponds.

Genetic parameters

The history of the GIFT strain has been described by Eknath and Acosta (1998) and by Bentsen *et al*. (1998). At the time the fish were received in Malaysia the GIFT strain had undergone six generations of selection. Asking whether there was evidence of diminishing additive genetic variance and of a plateauing response to selection in such a population would be legitimate questions. The results of our study indicated that there was still additive genetic variance for LW, the main focus of selection in the GIFT strain. Our heritability estimate (Table 6) was greater than that of Gall and Bakar (2002), but it was in good agreement with those reported by Kronert *et al*. (1989), and with the 'field environment' estimates of Oldorf *et al*. (1989). It was lower than the 'laboratory environment' estimates of the latter authors, and than those of Bolivar and Newkirk (2002).

Response to selection

Estimates of genetic gain per generation for aquatic animal species range from 10 to 20 per cent (Gjedrem 2000). In our case we may conclude that response to selection in LW between the 2002 and 2003 spawning seasons was of the order of 10 per cent. This falls at the lower limit of the range reported by Gjedrem (2000). It is also slightly lower than the response estimated by Gall and Bakar (2002), of 40 per cent in three generations. Our perception is that we could have achieved a greater response to selection if we had not suffered high (approx. 40 per cent) tag losses around harvest time. Tag losses caused a lower selection intensity and loss of the identity of many potentially valuable fish.

With regards to methodology, Chen and Boichard (2003) and Piles and Blasco (2003), working with poultry and rabbit data respectively, used an approach similar to ours in the estimation of response to selection. Overall, they found good agreement between the methods. In our case, the three methods used to estimate response to selection were also in reasonable agreement, and gave encouraging results. The agreement between methods (i) and (ii) was very good. Method (ii) gave a lower response than method (iii) because the average estimated breeding value in the progeny resulting from the 2002 spawning season was greater by about three percent than that in the Control line in the 2003 spawning season.

This suggests that there may have been a mild inadvertent selection for lower LW in the Control line. The lack of perfect agreement among different methods of estimation of selection response highlights the need to use alternative approaches to better interpret the results.

Conclusion

GIFT is an improved strain with proven growth potential (Dey *et al.* 2000). Our results indicated that despite having undergone several generations of selection, the population still has additive genetic variance to enable further improvement. This contention is supported by the selection response observed (10 per cent) after one round of selection in Malaysia. The response could be greater if tag retention to the point of harvesting could be improved.

In the initial exploratory analyses we found a statistically significant sire by environment interaction. This finding was a reason for some concern but, as is well known, such findings are not informative in terms of explaining the reason for the result (Robertson, 1959). In analyses conducted in addition to those reported here, we treated LW expressed in cage and in pond as different traits, examining the genotype by environment interaction with the genetic correlation approach, as indicated by Robertson (1959). The variance component due to interaction ($\sigma_{GE}{}^2$) can be partitioned into its contributing factors as:

$$\sigma_{GE}{}^2 = [(\sigma_C - \sigma_P)^2 + 2\sigma_C\sigma_P(1 - r_g)] / 2$$

where r_g is the genetic correlation between the expressions in both environments, and σ_C and σ_P are the between animal standard deviations of breeding values in cage and pond environment, respectively. The equation shows that the two contributing factors to the variance component due to interaction are the difference between the genetic standard deviations between the environments (scale effect) and a non-unity genetic correlation. A non-unity genetic correlation always results in a variance component due to interaction, but the opposite is not true. A variance component due to interaction may exist in the presence of a unity genetic correlation between the expressions of the trait in both environments, due to a scale effect. In our case, the non-unity genetic correlation was the almost sole contributor to the variance component due to interaction.

Falconer's (1952) approach of treating the expression of the trait in different environments as if they were different traits is helpful in understanding and drawing practical conclusions from the results. In our case, the genetic correlation between LWC and LWP was 0.58, indicating that if selection were conducted in one of the environments, about 60 per cent of the gain that could be achieved in the other environment would be captured. Our estimate of the genetic correlation had a relatively large standard error, resulting in 95 per cent confident limits ranging from 0.32 to 0.84. We also found that selection response separately calculated in cage and in pond environments was of the same magnitude as when LW was treated as a single trait. We will again estimate the genetic correlation and selection responses after adding the data of another generation, currently being reared. In the

meantime, we tentatively conclude that, despite finding a significant 'genotype by environment' interaction, there is not enough evidence to justify the conduct of separate genetic improvement programs for cage and pond environments in Tilapia.

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