GENETIC IMPROVEMENT OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) – PRESENT AND FUTURE

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Abstract

We mainly (but not exclusively) draw upon research and development work carried out by The WorldFish Center (WorldFish). We review the current state of development of selection programs that have had a main focus on growth rate and body traits. There is evidence of sustained gains of 10 to 15 per cent per generation over more than six generation. To date, these gains have not been accompanied by any undesirable correlated response. The prospects for altering sexual dimorphism and the shape of the fish appear to be very limited, however. We also examine the issue of the appropriate environment for selection. Not surprisingly, experimental evidence on genotype by environment interaction suggests that this is more likely to be of importance when the environments in question are markedly dissimilar. We argue that no universal guidelines can be prescribed regarding the need for more than one selection program to cope with different production environments, but that instead, each case should be examined in its own right. Finally, we discuss traits likely to be candidates for inclusion in future, more elaborate, breeding objectives for Nile tilapia, and comment on selection methods that may be implemented in the future.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is a well known tropical food fish native to Africa. To date several selective breeding programs for Nile tilapia have been established and maintained, for example GIFT (Eknath *et al.*, 1993; Eknath and Acosta, 1998), GET-EXCEL (Tayamen, 2004), FaST (Bolivar, 1998), and GST (GenoMar Supreme Tilapia, Zimmerman and Natividad 2004). These selective breeding programs have generally been implemented in Asia under relatively intensive culture systems where the fish are provided with formulated feeds. Nile tilapia has not been developed significantly in Africa with a focus on the prevailing production environments in the continent. This is despite the fact that Africa holds the global wealth of tilapia genetic resources and has a great natural potential for aquaculture development (Pullin, 1988).

In this paper we present information on the current state of development of selection programs that have had a main focus on growth rate and body traits

(including a study on the possibility of altering sexual dimorphism and shape of the fish), we examine the issue of the appropriate environment for selection, and we discuss traits likely to be candidates for inclusion in future, more elaborate, breeding objectives for Nile tilapia. We mainly (but not exclusively) draw upon research and development work carried out by The WorldFish Center (WorldFish).

SELECTION FOR GROWTH RATE

In this section we report in some detail the results derived from a Nile tilapia selection line jointly maintained by the Department of Fisheries (Malaysia) and WorldFish, for the period 2002 to 2007.

Origin of the fish. We have worked with the GIFT (Genetically Improved Farmed Tilapia) strain (Eknath *et al.*, 1993; Bentsen *et al.*, 1998; Eknath and Acosta, 1998), which has been disseminated to 11 countries in Asia (Gupta and Acosta, 2004). 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, and were received at Jitra, Kedah State, Malaysia, in batches towards the end of 2000 and during the beginning of 2001. They were mated and produced a seventh generation (Base population) 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), and another one selected for average breeding values (Control line). None of the parents used in one spawning season were used in a subsequent one (i.e. generations were discrete). Note that we consider the progeny produced in the 2002 spawning season our Base population, and in the analyses we treat it as part of the established Control line.

Overall objectives of the project. These may be summarized as follows:

1. To maintain and continuously improve the GIFT strain of Nile tilapia and to distribute it to partner countries likely to benefit from its use.
2. To conduct research that may enhance the effectiveness of the genetic improvement program (e.g. refining the methodology with respect to management of inbreeding and effective population size, introducing new traits such as fillet yield, flesh quality and response to thermal treatment to the breeding objective).
3. To utilize the data collected to support capacity building activities in the field of genetic improvement for staff from partner countries (because the GIFT population

maintained at Jitra is fully pedigreed it offers great opportunities for students and staff to conduct research on the data).

Update on phenotypic and genetic parameters and response to selection in GIFT. We have recently conducted a detailed analysis of all the data collected between since 2002. Tables 1 to 6 below summarize the results. Selection response was estimated for live weight. The data consisted of generation one to six bred in Malaysia and were collected at Jitra Research Station. Live weights were analyzed using the square root transformation.

Tables 1 and 2 show the number of observations by year, line (selection or control), sire and dam, and the work schedule, respectively. Numbers are such that they enable the estimation of parameters with confidence.

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
2003	Selection	35	65	2560
	Control	19	19	1150
2004	Selection	54	84	3714
	Control	17	22	957
2005	Selection	42	76	1763
	Control	13	20	480
2006	Selection	49	88	3638
	Control	10	15	591
2007	Selection	41	71	4238
	Control	15	15	859
Total		347	529	21634

Table 2.Schedule of reproduction and management.

Spawning Season	Activities					
	Mating	Nursing	Transfer to B-net	Tagging	Grow-out	Harvest
2002	Feb - Mar 02	Feb - Apr 02	Mar - May 02	Apr - May 02	Jun - Nov 02	28 Oct - 13 Nov 02
2003	Jan - Feb 03	Jan - Mar 03	Feb - Apr 03	Mar - Apr 03	Apr - Sep 03	18 Aug - 17 Sep 03
2004	Nov 03 - Feb 04	Nov 03 - Feb 04	Dec 03 - Mar 04	Feb - May 04	Mar - Sep 04	14 Aug - 22 Sep 04
2005	Dec 04 - Feb 05	Dec 04 - Feb 05	Jan - Mar 05	Mar - May 05	Mar - Sep 05	18 Aug - 08 Sep 05
2006	Nov 05 - Jan 06	Dec 05 - Feb 06	Jan - Mar 06	Mar - Apr 06	Mar - Sep 06	10 Aug - 04 Sep 06
2007	Oct 06 - Mar 07 *	Nov 06 - Apr 07	Dec 06 - May 07	Feb - Jun 07	Feb - Aug 07	14 Jun - 02 Aug 07

***** The prolonged mating period was due to not enough families produced for the control line in the first few weeks of mating.

Tables 3 and 4 show some simple statistics and the results of the analysis of variance, respectively. The coefficients of variation of both live weight and age at harvesting were lower in more recent years, suggesting that management during the grow out phase may have improved, thus resulting in a reduction of the variation in those two attributes. All effects fitted in the analysis of variance were statistically significant. Especially important in our case is the between line difference, since it indicates that there was response to selection. The significant spawning season by line by sex interaction can be explained by the fact that the between line difference became greater after each generation.

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

Variable	Spawning Season	N	Mean	Minimum	Maximum	Standard Deviation	Coefficient variation (%)
Live Weight	2002	1684	227.08	29.0	608.0	101.54	45
	2003	3710	154.11	7.0	617.0	86.27	56
	2004	4671	192.87	19.0	682.0	101.38	53
	2005	2243	209.38	75.0	551.0	59.32	28
	2006	3647	223.40	49.0	532.0	69.05	31
	2007	5097	222.15	15.0	504.0	73.93	33
Age at Harvesting	2002	1684	256.65	215.0	280.0	12.77	5
	2003	3710	213.94	125.0	265.0	25.28	12
	2004	4671	244.60	173.0	302.0	28.84	12
	2005	2243	227.48	182.0	260.0	14.52	6
	2006	3647	230.83	178.0	263.0	15.97	7
	2007	5097	219.93	118.0	272.0	19.71	9

Remark: The survival rate will also be analysed shortly. For the latest generation it was relatively high, at 82%, suggesting that the improvement in growth rate has not resulted in reduced fitness.

Table 4. Analysis of variance of $LW^{0.5}$: Tests of fixed effects using PROC MIXED in SAS.

Effects	F Value	Prob. > F
Spawning Season (SS)	5	< 0.0001
Line (L)	1	0.0109
Sex (S)	1	< 0.0001
Environment (E)	1	0.0257
SS*L*S	14	< 0.0001
Age (SS, S, E)	32	< 0.0001
Residual Variance	2.9601	

Remarks: This is the model with the best fit statistic (BIC: 84709.7). The random effects fitted were sire within spawning season and line, and dam within sire, spawning season and line.

Tables 5 and 6 show the genetic parameters and the selection response in live weight, respectively. The heritability was high, indicating that there is still abundant genetic variation and scope for further genetic improvement in growth rate. The maternal and common environmental effect (c^2) was large, and most likely due to the period in which the members of full sib families are growing together in hapas until each individual is large enough to be physically tagged. It would be desirable to eliminate or at least reduce the magnitude of this latter parameter.

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

Parameter	REML Estimate
Additive genetic variance (σ^2_A)	1.933
Maternal and common environmental variance ($\sigma^2_D = \sigma_{M\_Ec}$)	1.471
Phenotypic variance (σ^2_P)	5.57 (0.1694)
Heritability (standard error) [h^2 (s.e.)]	0.35 (0.0387)
Maternal common environment (standard error) [c^2 (s.e.)]	0.26 (0.0184)

Table 6 shows the selection response in growth rate experienced by GIFT in Malaysia. Here we only comment on the response expressed in percentage terms. There was continued response over the period examined, as well as good agreement between the two methods used (32.7 per cent comparing estimated breeding values in consecutive generations vs 32.9 per cent comparing the selected and control lines). Note that these percentages were calculated from results using the square root transformation on the data. James (2007) shows that the percentage selection response after square root transformation is a fraction 0.501 of that in actual units. This means that in actual units the response for the period in question was of the order of 65 per cent (an average of 13 per cent per generation). Coupled with the high survival consistently observed in the GIFT strain, the high potential for growth rate makes it a very attractive genetic resource.

Table 6. Response to selection estimated by different methods and expressed in different ways.

Method		Model (effects)	Selection Response ($LW^{0.5}$)[A]		
			Actual units ($g^{0.5}$)	Per cent	Genetic standard deviation units (actual/σ_A)
(i) Comparing the estimated breeding value for live weight between progeny from selection line of two consecutive spawning seasons.					
a.	Between 2002 and 2003 spawning season.	Fixed: SS x L x S x E Covariate: Age at harvest (SS, L, S, E) Random: Spline (age_hv), uni (sex,2), animal and DAM	1.237	7.745	0.890
b.	Between 2003 and 2004 spawning season.		1.149	7.199	0.826
c.	Between 2004 and 2005 spawning season.		1.043	6.533	0.750
d.	Between 2005 and 2006 spawning season.		0.769	4.816	0.553
e.	Between 2006 and 2007 spawning season.		1.029	6.446	0.740
(ii) Comparing the estimated breeding value for the live weight between progeny from control line and selection line of the same spawning season.					
a.	2003 spawning season	Fixed: SS x L x S x E Covariate: Age at harvest (SS, L, S, E) Random: Spline (age_hv), uni (sex,2), animal and DAM	1.590	9.959	1.144
b.	2004 spawning season		2.803	17.553	2.016
c.	2005 spawning season		3.595	22.516	2.586
d.	2006 spawning season		4.214	26.391	3.031
e.	2007 spawning season		5.253	32.898	3.778

[A] Actual units are $LW^{0.5}$ difference in mean breeding values for methods (i) and (ii); percentage refers to actual units, in relation to the least squares means of $LW^{0.5}$ for the control population ($15.9665 g^{0.5}$); genetic standard deviation equals the square root of the additive genetic variance in table 6 ($\sigma_A = 1.3903 g^{0.5}$).

FURTHER EVIDENCE OF GENETIC GAIN IN GIFT

In a separate experiment (Khaw *et al.*, 2008b) genetic change in GIFT was estimated by comparing the performance of the progeny produced from cryopreserved spermatozoa from the base population with that produced by freshly collected spermatozoa from the ninth generation. The comparison involved artificial fertilization of 13 males from each generation (base and ninth) with a random sample of female brood stock. The progeny produced went through a 120 day grow-out period, after which live weight, standard length, body depth and survival were recorded. The

estimated total genetic change in live weight was 64 per cent over nine generations, or 7.1% generation (Table 7). The genetic change was lower than the estimate reported by Eknath *et al.* (1998), but in the present experiment the time span included generations in which there was no selection. The improvement in the latter trait was achieved without any deterioration in survival rate, which has remained high.

After five generations of selection, Eknath *et al.* (1998) reported an annual genetic gain of 12-17%, which is considerably higher than our estimate for nine generations. Eknath *et al.* (1998) did not establish a separate control population and maintain it throughout these five generations, but rather, they recreated a new control in each generation by mating a sample of average individuals for that generation. There may have been inadvertent selection of the fish to be mated as controls, and sampling problems accumulated during the selection could have caused an over estimation of the genetic gain that reported by Eknath *et al.* (1998).

By contrast, the estimate of genetic gain from Khaw *et al.* (2008b) may actually be biased downwards. Firstly, the frozen sperm was collected from the best growing males in the base population, whereas the sires used from the ninth generation were not the best (they were close to the average of that generation). Secondly, the formal GIFT selection program ended at the fifth generation, and there was no selection when matings were conducted to produce the sixth and seventh generations. The sixth generation was sent to Malaysia and was used there to establish the population now located in Jitra Aquaculture Extension Center, Kedah state. That means in between these nine generations, there are two in which no selection of superior individuals for growth rate or any other trait was conducted. If allowance were made for this when calculating genetic gain per generation, a figure (9%) closer to that reported by Eknath *et al.* (1998) would be obtained.

In Africa, where very little has been done in terms of genetic improvement of Nile tilapia, one may safely assume that the productivity of the current stock is at the level of the GIFT base population or lower (Brummett *et al.*, 2004). Hence, one may also safely assume that the introduction of GIFT to Africa would improve growth by at least 64%. This is not a trivial gain, and it could greatly benefit emerging aquaculture industries in many African countries.

Table 7. Harvest weight and survival rate least squares means and standard errors (s.e.) for sire generation (means with a different subscript are significantly different ($P<0.05$)).

Sire generation	Harvest Weight (g)	Survival (s.e.)
Base	141_a (5.8)	0.86 (0.02)
Ninth	186_b (5.8)	0.83 (0.02)

CHANGING SEXUAL DIMORPHISM AND THE SHAPE OF THE FISH

In Nile tilapia males are of greater size than females. In terms of fish shape preferences may vary, but in any case ascertaining whether relative body dimensions may be altered by selection is of interest. Using the Malaysian GIFT data set we conducted a study with the aim of estimating genetic parameters, especially focusing on the genetic correlation between trait expressions in both sexes and among measurements of body size (Nguyen *et al.*, 2007). Body weight, length, depth and width data at harvest from 12,308 individuals, progeny of 232 sires and 340 dams were analyzed by restricted maximum likelihood methods fitting a multi-trait animal model. To explore the genetic variation in sexual dimorphism the trait expressions in the two sexes were treated as if they were different traits. Heritabilities and maternal and common environment effects for all the traits were very similar in males and females. The genetic correlations between sexes for all traits were close to unity (0.91 to 0.96), indicating that there was no sex by genotype interaction. When treated as a single trait the heritabilities (± SE) for body weight, length, depth and width were moderate to high, ranging from 0.20 to 0.35 (± 0.04 to 0.05). The maternal and common environment effects accounted for 16 to 24 per cent of the variance. Genetic correlations among the four body measurements were highly positive (0.94 to 0.99), suggesting the existence of little or no genetic variation independent of each other. Nguyen *et al.* (2007) concluded that there was no need to treat trait expressions in the two sexes as different traits in genetic improvement programs. Furthermore, that the relative dimensions of the body were essentially controlled by the same genes, but that continued selection for live weight would result in relatively longer and thinner fish because of the greater correlated response in length relative to width and depth.

In conclusion, because of the very high genetic correlation between the expressions of body traits in the two sexes, in GIFT there is very limited scope for selection for sexual dimorphism. With regards to body shape there is also limited scope for change, but the results obtained with all the selection alternatives (indices combining harvest weight, length, width and depth) that were examined indicate that selection for greater harvest will slowly result in relatively longer and thinner fish due to greater response in length than in width and depth.

ENVIRONMENT FOR SELECTION

Traditionally, tilapia has been cultured in earthen ponds under extensive and semi-intensive systems (El-Sayed, 2006). Due to the shortage of freshwater and wide spread distribution of tilapia culture, farmers and commercial entrepeneurs have in many cases shifted to more intensive systems in ponds, tanks and cages (El-Sayed, 2006). With the wide range of culture systems and environments between countries

and within a country (especially for large countries, such as China, or a whole region such as Sub Saharan Africa) ascertaining the appropriate environment for selection is important. Furthermore, during last two decades, improved Nile tilapia strains, for example, GIFT (Eknath *et al.*, 1993; Eknath and Acosta, 1998), GET-EXCEL (Tayamen, 2004) and FaST (Bolivar, 1998), have been widely distributed from Asia (the continent where those fish were selected) to other parts of the world. These two features of present tilapia production clearly indicate the need for genotype by environment interaction (G x E) studies in Nile tilapia breeding programs.

Several G x E studies have been carried out in different countries. In Malaysia, the growth performance of GIFT was investigated in two main culture environments, earthen ponds and cages. This study (unpublished authors' results) shows that the genetic correlation for harvest weight between pond and cage was 0.70±0.11. In another G x E study with GIFT conducted in Philippines seven different environments encompassing a range of farming systems and agro-climatic regions were examined (Eknath *et al.*, 2007). Live weight expressions in all the environments were positively correlated with each other, and the correlations ranged from 0.36 to 0.99. The correlations were generally high within the pond environments (0.76 to 0.99) and within the cage environments (0.99), but they were lower in some cases and more variable between the pond and cage environments (0.36-0.82). These results illustrate the notion that the greater the difference between environments, the greater the chance of finding an important G x E.

This latter concept is supported by a recent G x E study with GIFT by Luan *et al.* (2008) in Vietnam. They studied harvest weight and survival of GIFT growing out in brackish and in freshwater systems. The genetic correlations between these two environments are 0.45±0.09 and 0.42±0.05 for harvest weight and survival, respectively. The authors conclude that given these correlation values two separate selection programs should be undertaken for the improvement of these traits in brackish and in freshwater environments.

Khaw *et al.* (2008a) carried out a G x E study with Nile tilapia at the Regional Research Station of The WorldFish Center at Abbassa, Egypt. From the same brood stock, two selection lines were developed: one in which all the progeny were reared in a low input environment (only received chicken manure as a daily external nutrient source), and another one maintained in a high input environment (received formulated pellets that contained 25 percent protein). The data set consist of 7,640 animals (progeny of 298 sires and 493 dams) with body weight records at harvest over three discrete generations. With regards to the statistical analyses to examine the G x E, four different models (with different fixed effects) were fitted in a bivariate animal

mixed model to estimate the relevant genetic parameters (harvest weight in each environment was treated as a different trait). The genetic correlation for body weight between the low and high input environments ranged from 0.74 to 0.84 (with a standard error of 0.15-0.36). Unfortunately, Khaw *et al.* (2008a) found some degree of confounding between genetic and environmental effects. This is mainly due to limitations in the data structure (discrete generations and without a control line), which did not enable a neat separation of genetic and environmental effects from each other.

Robertson (1959) suggested that when the genetic correlation between the trait expressions in two different environments was greater than 0.8 the G x E could be considered unimportant. However, no universal guidelines for the handling of G x E in animal breeding have been developed, and due to the nature of the problem, quite likely, never will. Thus, we should treat every case based on its nature, merits and economic aspects (James, 2008). For example, in the study by Khaw *et al.* (2008a) that was carried out in Egypt, the lower limit for the genetic correlation for harvest weight in both environments was smaller (0.74) than 0.8, which following Robertson's suggestion would indicate that two different selection programs would be required. Note however, that, for instance, if the selection program were conducted in the low input environment, the genetic correlation indicates that 74 per cent of the gain would be captured in the high input environment. Due to the fact that Egypt is a developing country with limited resources, in this situation justifying having two breeding programs for one species would be difficult. In such circumstances, the decision should be made weighing the statistical evidence on G x E, the likely future evolution of the environmental conditions, and economic aspects of the country in question in relation to resources available for genetic improvement programs (Montaldo, 2001).

In the future, with the rapid expansion of the Nile tilapia aquaculture industry, more in depth G x E studies should be carried out by focusing on growth performance and survival rate in a range of different realistic culture environments (e.g. ponds, cages and tanks), culture systems (e.g. extensive, semi-intensive and intensive) and agro-climatic environments (e.g. brackish and freshwater, tropical, sub-tropical and temperate, highly and lowly seasonal) likely to prevail in the foreseeable future.

PRODUCTION OF ALL MALE POPULATIONS FOR GROW OUT AND MARKETING

Producing 'all male' tilapia populations for the production system. Monosex culture of male tilapia is often preferred to the mixed sex system due to sexual dimorphism, males being substantially larger than females (e.g. Ponzoni *et al.*, 2005, Nguyen *et al.*, 2007). In addition, precocious reproduction of this species also leads to low growth performance of stocked fish as a consequence of over-crowding and feed

competition in production ponds. There are several methods available for the creation of monosex tilapia populations for the production system, such as manual sexing, interspecific hybridization, androgenesis, triploidy, transgenesis, hormonal sex reversal, and YY male technology (Beardmore *et al.*, 2001). However, the efficiency of these technologies varies with populations, seasons of fry production and culture environments. To date, the available 'all male' production methods have suffered from one or more limitations that make them either not cost-effective, unsustainable or not acceptable. For instance, masculinizing tilapia with hormones has raised consumers' concerns regarding food safety. In the case of YY technology, the creation of YY males takes three generations of breeding. This means that if the source population is undergoing selection, by the time the YY males are ready for use they will be lagging three generation behind in terms of genetic gain. In practical terms this could be between 20 and 45 per cent, depending on the rate of progress in the selected population. Furthermore, the application of YY technology relies upon the participation of a laboratory with relatively advanced facilities for the production of YY males. It creates a relation of dependence of the hatchery producing the all male progeny for the production system on the laboratory providing the YY males. This is not a scenario to be favoured in general and even less so in developing countries. Other techniques also have limitations that we do not discuss here. Hence, alternative means of producing all male tilapia populations merit further research (e.g. increasing male to female ratio in response to thermal treatment).

A novel method: thermal treatment. Production of monosex tilapia (males) can be achieved with a high rate of success by temperature treatment (e.g. Baroiller *et al.*, 1995; Baroiller and D'Cotta, 2001). Across studies, a high temperature between 36 and 38 ^{o}C applied over 10 days post-hatching (the critical sensitive period of sex differentiation) can induce a high proportion of males (above 80%). A number of studies suggested possible temperature by genotype interaction (Baroiller *et al.* 1995; Abucay *et al.* 1999; Baroiller and D'Cotta, 2001, Tessema *et al.* 2006). There is also a large between family (within population) variation in the level of thermo-sensitivity to the treatment, as recently demonstrated by Tessema *et al.* (2006) and Bezault *et al.* (2007). In summary, there is potential for masculinization of female genotypes by temperature treatment. However, high temperatures can also have both masculinizing and feminizing effects, depending on the sex genotype, origin of the fish, and various genetic and environmental factors affecting the sex determination mechanisms.

At The WorldFish Center, we have conducted preliminary studies to investigate the effect of heat treatment on sex ratio, growth and survival in the GIFT strain. Experiments with the application of various levels of temperature at different rearing

periods have been conducted under both laboratory and field conditions. In each experiment, the newly hatched fry of each family (soon after egg yolk absorption) was split into two or three groups of equal size. One group was reared under normal water temperature between 28 and 30 ^{o}C (control). The temperature treatments were applied to other groups for which water temperature was set at 36^{o}C or at 38^{o}C and kept constant by special heaters for 10 and 6 days, respectively. After the treatment period, the fry of each family were returned to normal water conditions in separate hapas installed in tanks, or transferred to nursery hapas in ponds. All groups were subject to the same feeding and management regimes throughout the experimental period. Under the laboratory test, fingerlings of an average size of 4 g were sexed using the aceto-carmine staining method. Manual sexing was conducted with the fish under field conditions. At sexing, measurements of body weight and standard length were recorded for individual fish. Across the experiments, a chi-square test showed slightly greater male to female ratio in the treatment groups than in the control. There was variation in sex ratio among families, ranging from 20 to 80% of males in some families. For body traits, there were no statistical differences in body traits between the control and heat treatment groups. Survival of fry from hatching to sexing was in general lower in the heat treatment group than the control. A new series of replicate experiments is now underway. The principal aim of this study is to define a suitable experimental protocol to increase male to female ratio in the GIFT strain by thermal treatment. If the GIFT strain responded to the heat treatment as reported in other studies, there would be possibilities of conducting selective breeding for sensitivity to the treatment. In a recent study conducted by Wessels and Horstgen-Schwark (2007), two generations of selection resulted in a male percentage of 90%, with a realized heritability of 0.69.

In addition to conventional selective breeding to increase male to female ratio, in the future, if novel genes with large effects were successfully detected, a combination of marker assisted selection and polygenic selection could significantly increase the rate of response for thermo-sensitivity. The outcome of such a study could help provide a practical and totally acceptable technology for sex control in tilapia, and perhaps for other fish as well. Genetically divergent selection lines developed from conventional genetic selection could be used for subsequent studies on physiological and molecular genetic mechanisms of sex determination in this strain. Some studies have characterized genes (e.g. MM20C that is differentially expressed in temperature masculinized females (D'Cotta *et al.*, 2001) or CYP19 genes (Chang *et al.,* 2005) that encode aromatase cytochrome P450 enzyme). The P450 enzyme catalyzes the formation of oestrogens from androgens. A suppression of aromatase gene

expression can result in masculinization associated with a functional testis development in tilapia. However, the regulation of aromatase genes is presently not well understood. Thus one important component of a study in the area of genomics would be to focus on the identification of quantitative trait loci (QTL) and candidate or functional genes associated with sex ratio in GIFT. The successful detection of major genes would increase the rate of genetic progress through a combination of marker assisted selection (MAS) with conventional selective breeding.

Finally, the success in improving growth rate by selective breeding has somewhat reduced the importance and perceived need to have all male tilapia populations in the production system, unless the aim is to market the fish at live weights greater than 800 g. The market size of the fish in most tilapia production countries normally ranges from 200 to 600 g. Within this range, performance of GIFT is little affected by sexual maturation because selection has remarkably increased growth rate of the fish (e.g. achieving 600 g within three months of culture under a standard farming environment in Malaysia (Azhar Hamzah, pers. comm.). From a management point of view, minimizing variation in body traits can be done through improved management practices such as synchronization of fry production, improved nutrition and reduced stocking density.

FILLET YIELD AND FLESH QUALITY

Consumers and the aquaculture industry are increasingly focusing their attention on flesh and eating quality. In some countries, the marketing system has shifted from payment based on whole fish live weight to fillet weight. There is thus a need to examine the possibility of broadening the breeding objective in GIFT and other strains to include these additional traits.

Strain comparisons. Red tilapia is a preferred strain in Malaysia with a reputation of having very good flesh quality. Khaw *et al.* (2006) and Ponzoni *et al.* (2006) carried out a series of sensory evaluation studies on the flesh quality and fillet traits of GIFT and red tilapia. Regarding the flesh quality studies, the results show that although there were significant differences in some sensory attributes between GIFT and red tilapia, these were not consistent (differed between tests, did not consistently favor one of the strains). However, the more important finding was that the score for all the sensory attributes for both strains fell within the range of highly acceptable flesh. The analysis of fillet traits showed a similar pattern in all the experiments conducted (Khaw *et al.*, 2006; Ponzoni *et al.*, 2006). GIFT emerged as a valuable strain for filleting, but not due to an advantage in fillet yield (which was very similar between both strains), but because of its greater fillet weight due to its greater growth rate. The fillet yields reported in the studies by Khaw *et al.* (2006) and Ponzoni *et al.* (2006)

ranged from 26.66% to 38.89%, and they are in agreement with the values reported by Rutten *et al.* (2004), which are in the range of 26% to 37%. From their study on flesh quality and fillet traits Khaw *et al.* (2006) and Ponzoni *et al.* (2006) conclude that GIFT is a high performing strain in terms of growth rate and fillet traits. Although no important between strain differences were found in the sensory evaluation, it can be concluded that GIFT is comparable to Red tilapia (the preferred strain in Malaysia) in that respect, hence totally acceptable by Malaysian consumers, which makes it a strain of great potential for both the domestic and the export markets.

Within strain estimation of phenotypic and genetic parameters. We have initiated a study examining the following traits: i) Fillet weight and yield, ii) Carcass composition, and iii) Fatty acid composition. The data were collected over three generations from the long term selection program in GIFT tilapia. During 2006, 2007 and 2008 data on fillet weight and yield were recorded in about 5500 fish from more than 100 full sib families (Table 8). Fillets were weighed and frozen for later use in the assessment of flesh quality attributes. Approximately 2000 fillets were sent to a specialized laboratory for the analysis of flesh quality attributes, such as protein %, moisture %, fat %, pH and color (Table 9). A sub-set of the samples were also analyzed for fatty acid composition (Table 10).

Table 8. Body traits at slaughter and fillet weight and yield

Traits	Unit	Mean	Animals	Sires	Dams
Fish weight	g	527.0	5332	169	276
Length	cm	23.8	5332	169	276
Width	cm	10.3	5332	169	276
Depth	cm	4.7	5332	169	276
Fillet Weight	g	177.7	5332	169	276
Fillet Yield	%	33.6	5332	169	276

The fillet yield of GIFT tilapia was about 34%. This is greater than the average value reported in the literature for the species, which ranges from 25 to 35% depending on population, age of the fish, slaughter weight and method used to determine the yield (reviewed by Nguyen et al., unpublished results).

Table 9. Carcass composition and fresh quality of GIFT tilapia

Traits	Unit	Records	Mean
Protein	%	2034	20.95
Fat	%	2034	0.83
Moisture	%	2034	78.61
pH	Unit	2034	6.23
Color*	Score	2034	2.45

*color scale: 1 (white), 2 (grey), 3 (orange), 4 (pink) and 5 (red)

Table 10. Fatty acid composition (%) in GIFT tilapia frozen fillet

Type	Fatty acid	Common name	No. of samples	Mean
SFA	C6 : 0	Caproic acid	514	0.13
	C8 : 0	Caprylic acid	514	0.22
	C10 : 0	Capric acid	514	0.36
	C12 : 0	Lauric acid	514	0.19
	C14 : 0	Myristic acid	514	3.46
	C15 : 0	Pentadecanoic acid	514	0.31
	C16 : 0	Palmitic acid	514	26.81
	C17 : 0	Heptadecanoic acid	514	0.49
	C18:0	Stearic acid	514	5.88
	C20 : 0	Arachidic acid	514	0.34
	C22 : 0	Behenic acid	514	0.19
	C24 : 0	Lignoceric acid	514	0.14
	Total SFA			37.83
MUFA	C16 : 1 n-7	Palmitoleic acid	514	6.43
	C18 : 1 n-9t	Elaidic acid	514	0.43
	C18 : 1 n-9c	Oleic acid	514	30.40
	C20 : 1 n-11	Eicosenic acid	514	1.07
	C22 : 1 n-9	Erucic acid	514	0.10
	Total MUFA			38.31
PUFA	C18 : 2 n-6t *	Linolelaidic acid	514	0.40
	C18 : 2 n-6c *	Linoleic acid	514	10.21
	C18 : 3 n-6 *	g-Linolenic acid	514	0.63
	C20 : 5 n-3 **	Eicosapentaenoic (EPA)	514	0.48
	C22 : 6 n-3 **	Docosahexaenoic (DHA)	514	3.15
	Total PUFA			14.69
Others				9.17

SFA (Saturated fatty acid), MUFA (Monounsaturated fatty acid), PUFA (polyunsaturated fatty acid),

* Omega-6, ** Omega-3

Fatty acids can be categorized into three main groups (saturated fatty, monounsaturated fatty and polyunsaturated fatty acids) (Table 10). Unsaturated fatty acids, especially omega-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3) are widely recognized to be beneficial for human health and nutrition. They are associated with biochemical processes involved in the prevention and reduction of cardiovascular diseases, diabetes, rheumatoid arthritis and cancers. Fish are generally rich in the long chain omega-3 fatty acids. Levels of EPA and DHA in GIFT tilapia fillets were lower than in marine species, but they were consistent with the results reported in the literature for tilapia (e.g. Bahurmiz and Ng, 2007; de Souza *et al.*, 2007) or for other freshwater fishes (de Castro *et al.*, 2007). The levels of SFA and MUFA in GIFT fillets were also similar to the values reported in the literature. However, a rigorous comparison among studies is impossible because fatty acid composition depends on several factors such as stock origin, culture environments, and especially diet.

A full account of the results from these studies will be published in the scientific literature in the near future. The work will include modeling of fillet yield based on body measurements, estimation of phenotypic and genetic parameters for carcass and flesh quality traits and fatty acid composition and their relationship with production performance in GIFT.

CONCLUSIONS

The WorldFish Center has played a pioneering role in the initiation and conduct of genetic improvement for aquatic species in developing countries. This leading role should in many cases continue. We approach work in this area in a logical and systematic manner, by addressing, as deemed appropriate in each circumstance, all the activities that the planning, design and conduct of a genetic improvement program entail, namely:

1. Description or development of the production system(s)
2. Choice of the species, strains and breeding system
3. Formulation of the breeding objective
4. Development of selection criteria
5. Design of system of genetic evaluation
6. Selection of animals and of mating system
7. Design of system for expansion and dissemination of the improved stock
8. Monitoring and comparison of alternative programs

This approach is not only useful in itself in the sense that it enables a logical treatment of the subject matter, but it is also helpful in the identification of areas in which knowledge or its application are deficient, and that should therefore become the

target of research, development and technology transfer. As the genetic improvement programs unfold, limitations and areas where there is room for refinements are identified. These, then become focused research areas, the results from which feed back into the program making it gradually more effective.

At present, especially in newly initiated genetic improvement programs, growth is likely to continue to be the main focus of selection. This is justified because of the great economic importance of the trait in the production system given the very high reproductive rate of fish. Most developing countries completely lack improved strains, so initially, even despite possible evidence about G x E, a single program will have to service a range of production environments. Later, as the program progresses, gets consolidated, and production systems get better defined, resources permitting, specialized programs servicing specific production systems may be developed. With the development of more sophisticated markets, either domestic or export, the need to select for fillet yield and flesh quality traits may emerge. This will add complexity to the programs and inevitable fully pedigreed populations and BLUP (Best Linear Unbiased Prediction) evaluations for the estimation of genetic merit of such traits will have to be conducted. Selection for less conventional traits, such as sensitivity to thermal treatments with the purpose of producing all male progeny, may have to be integrated as well to increase the efficiency of some production systems. Selection for disease resistance has not emerged as a priority in tilapia, but it may in the future as production systems intensify. The classic selective breeding technology based on quantitative genetics can serve selection programs for growth rate and survival well, but for the more difficult to measure traits such as carcase and flesh quality, sensitivity to thermal treatment and disease resistance, molecular techniques would be very valuable if they enable selection on markers or directly on the genes affecting the trait. Our perception is that in the foreseeable future simple programs totally based on quantitative genetics will co-exist with much more sophisticated ones using quantitative genetics jointly with state of the art molecular genetics techniques, each program effectively servicing a specific set of production, economic and social conditions.

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