

PERFORMANCE AND SURVIVAL OF THREE RED TILAPIA STRAINS (*OREOCHROMIS SPP*) IN POND ENVIRONMENT IN KEDAH STATE, MALAYSIA

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Abstract

Growth performance (body weight, standard length, body width and depth) and survival of three red tilapia (*Oreochromis spp*) strains from Malaysia, Taiwan and Thailand were evaluated using 2,867 records collected on individually tagged fish after a period of three-months of communal rearing in an earthen pond. The statistical model used to analyze the data included strain, sex and their two-way interaction as fixed effects and age from stocking to harvest and initial weight as linear covariates. The effect of strain was highly significant for all body traits ($P < 0.001$). The Malaysian strain had the fastest growth rate, whereas the lowest performing strain was that from Thailand. The growth performance of the Taiwanese strain was intermediate. Across strains, body measurements of red tilapia females were significantly ($P < 0.001$) smaller than those of males. The strain by sex interaction was statistically significant ($P < 0.05$ to 0.001). We also analyzed survival rate of the fish from stocking to harvest, using a generalized non-linear model. Survival was considered as binary trait, and the model assumed that the data followed a binomial distribution, with a logit link function ($p^{\wedge} = e^x / (1 + e^x)$). In contrast to the results for body traits, survival rate was poorest for the Malaysian strain (66.4%). The difference in survival rate between Taiwan and Thai strains (78.5 vs. 80.6%) was not statistically significant ($P > 0.05$). Our results suggest that there are genetic differences in growth performance and survival among red tilapia strains from Malaysia, Taiwan, and Thailand. They are thus being included in a complete diallel cross to further examine the additive and non-additive genetic performance of the strains before forming the base population for future genetic selection. In this paper we present an outline and key activities of the planned genetic improvement program for red tilapia in Malaysia.

INTRODUCTION

In Malaysia, red tilapia contributes approximately 90% of the total tilapia production. The production of red tilapia increased from 8,214 tonnes in 1998 to 20,061 tonnes in 2003, with an expansion rate of 144% during this period (Annual

Fisheries Statistics of Malaysian Fisheries Department, 1998; 2003). The species *Oreochromis spp* in general is widely cultured in ponds, cages and tanks as well as in pen culture systems. By the year 2010, tilapia production is targeted at 150,000 tonnes. Large water bodies such as lakes, reservoirs, ex-mining pools and irrigation canals available are also potential production areas for cage culture of this species.

Aquaculture business in the country has involved many fish farmers and commercial companies, practicing several types of production systems. However, they almost exclusively use unimproved species and strains. It seems that currently available knowledge and experience in aquaculture systems management are not being fully exploited to achieve sustained yield and optimum fish performance. In this context, the development and use of genetically improved stocks that are more productive becomes imperative in order to more effectively utilize the resources. Hatchery operators and fish farmers have used the available tilapia strains without information on their history, background, performance and pedigree information. Improper brood stock management associated with high level of inbreeding has also resulted in a decline in productivity of cultured populations under various commercial settings. Periodically, tilapia brood stocks have also been imported into Malaysia by private hatcheries. Crossbreeding and hybridization are mostly practiced to improve the strains performance. Traits such as growth, survival rate, body conformation and colour are of primary concern in producing new hybrids.

Because of the above mentioned issues and the great production potential of red tilapia in aquaculture, there is a need to develop a genetically improved strain for the species in order to meet growing demand for high quality seed by fish farmers in the country. Selective breeding programs have proved their positive impacts in aquaculture development and poverty alleviation especially in Asia and Africa. Successful examples of tropical freshwater improved fish strains include GIFT tilapia (Genetically Improved Farmed Tilapia, *Oreochromis niloticus*) and Indian 'Jayanti' rohu carp (*Labeo rohita*). Generally the genetic improvement program in red tilapia will follow the GIFT methodology (e.g. Ponzoni *et al.*, 2005). Steps include population sampling, strain evaluation, establishment of the base population, conduct of the selection program, and design and refinement of the breeding procedures.

As a first step, this experiment was carried out with the aim to evaluate growth performance and survival of three red tilapia strains from Malaysia, Thailand and Taiwan. The growth performance (body weight, standard length, body width and depth) and survival rate were evaluated using 2,867 records collected on individually tagged fish after a period of three months communal rearing in an earthen pond. Based on performance ranking, the best parents of each strain are being selected to

be included in a complete diallel cross to further examine the additive and non-additive genetic performance of the strains. The subsequent step will be to form a synthetic base population for future genetic selection.

MATERIALS AND METHODS

Fish origin

This study was carried out at the Aquaculture Extension Centre in Jitra, Kedah State, Malaysia. The experimental fish were three red tilapia strains i.e. Malaysia, Taiwan and Thailand. The Malaysian strain was produced at Freshwater Fisheries Research Centre in Jelebu, Negeri Sembilan State, and then was transferred to Jitra in Kedah. The Taiwan and Thailand strains were obtained from Pathumthani Fisheries Test and Research Center (PFTRC), Aquatic Animal Genetics Research and Development Institute, Thailand. The origin of the Thai strain is given by Pongthana *et al.* (2008, submitted).

The Malaysian strain was formed by the fish from four locations: Negeri Sembilan, Enggor, Lorong Pandan and Bentong. Negeri Sembilan and Enggor were ranked at the top in a strain evaluation experiment involving four stocks in Jitra. A parallel experiment at the Freshwater Fisheries Research Center (FFRC) in Jelebu also selected the best two stocks (among 12) from Lorong Pandan and Bentong. These four stocks were crossed in a complete diallel design. Mating was carried out in two batches at FFRC. Progeny at an average size (standard length) of 3 cm from the first batch of the diallel cross were sent to Jitra in June and the second batch in September 2007, with a total of 20 and 18 families, respectively. Families were individually reared in hapas, at a stocking density of 100-200 fry per m². A high mortality occurred in some families; however, there were still sufficient numbers of fish to be tagged per family. Due to three-month interval between batches, only fish of the first batch were used in this experiment, although the program utilizes all available families and stocks in order to capture maximum genetic variability in the base population for future selection.

In parallel with the arrival of the fish from FFRC, a total of about 2400 fry of the Thai and Taiwan strains were also brought to Jitra Research Station in Kedah, Malaysia. Progeny of each strain were produced from 100 female and 100 male parents through mass spawning in separate 50 m³ cement tanks at PFTRC. The average weight of female and male parents were 733.3 g (SD=190.8 g) and 746.4 g (SD=173.4 g) for the Thai strain, and 922.3 g (SD=168.1 g) and 886.6 (SD= 202.4 g) for the Taiwan strain, respectively. Age of the fry sent to Jitra was about 15 days old. At that age, they were about 1.5 cm. After arrival in Jitra, all the strains were quarantined for two weeks before transferred to the nursery hapas.

Nursery

After the quarantine period the fish were transferred into their respective nursery hapas until they reached about 5 g average body weight. All the hapas were installed in the same pond to minimize environmental effects. Stocking density in the hapa for each strain was 80 fry/m². The hapas were covered with a net to prevent access by birds which can cause mortality during nursing. Feeding at a rate of 5% of their biomass was practiced twice a day. Water parameters such as dissolved oxygen, pH and temperature in the nursery pond were monitored every three days.

Tagging

Once the fry average weight reached about 5 g, tagging was conducted to identify the strains and the individuals within each strain. PIT (Passive Integrated Transponder) tags were used for this purpose. Fifty fry from each family of the Malaysian strain were randomly collected and tagged, whereas 1,000 fry were randomly sampled from the Taiwan and from the Thailand strains for individual tagging. All the tagged fry were transferred into three 5m x 2.5m x 1m conditioning hapas for three days in order to monitor mortality. The dead or tag lost fry were re-tagged and replaced by their siblings from the same strain during that period. At tagging, the identification code, body weight, standard length, body width and depth of each fry were recorded. All the tagged fish from the three strains were then released into an earthen pond for communal grow-out.

Test environment

Prior to tagging, a 30m x 30m x 1.1m earthen pond was prepared based on a standard pond preparation method. All the tagged fry were released in the pond at day 4 after tagging. Standard culture management was applied during the grow-out period, and the fish were fed twice daily a standard commercial pellet containing 32% protein content at the rate of 3-5% body weight. Water parameters such as dissolved oxygen, pH and temperature in the pond were monitored every three days.

Harvesting and data collection

At the end of the grow-out period (approximately 130 days), all the cultured fish were harvested for performance evaluation in early April 2008. Feeding was stopped two days prior to harvest to reduce stress. A seine net was used in the ponds by seining in two drags. The pond was completely dried in the following day, early in the morning. All the fish were transferred to the conditioning cages (3m x 3m x 1m) installed in another pond. Data recording was done three days after the conditioning period. Measurements were taken of body weight (BW), standard length (SL), body width (WD) and body depth (DP). Width and depth were measured at the mid-side of the fish, where they were greatest. The tag number and sex of individual fish were

also recorded. Sex evaluation was done by visual assessment of the genital papilla. During the culture period almost no fish lost their tags. This enabled the statistical analysis of survival from stocking to harvest. After recording, the fish were transferred back to their conditioning cages until data analysis was completed.

Statistical analysis

All analyses were carried out using SAS (SAS Inc, 1997). For body traits, we used a general linear model (GLM) to identify the factors to be included in the statistical models. The model used to analyze body traits included the fixed effects of strain, sex and their first order interaction, and the covariables of age at harvest and initial body weight of the experimental fish. All the fixed effects and covariables were highly significant ($P < 0.001$) for body traits (except age for body depth, $P < 0.05$). A summary of the analysis of variance (ANOVA) for body traits is shown in Table 1. The R^2 values of the full model ranged from 0.30 to 0.36 for the observed traits. In order to examine relative importance of individual effects, we calculated partial R^2 by excluding one effect at a time from the full model.

Table 1. Analysis of variance

Effect	Weigh			Leng		Width		Dept	
	DF	F-value	R ²	F-value	R ²	F value	R ²	F-value	R ²
Strain	2	37.2***	0.25	26.4***	0.15	18.2***	0.21	28.5***	0.23
Sex	1	86.5***	0.04	96.8***	0.05	144.2***	0.05	47.1***	0.03
Strain*sex	2	12.5***	0.01	14.3***	0.01	3.7*	0.00	4.4***	0.01
Age	1	47.6***	0.02	69.4***	0.03	78.6***	0.02	36.7*	0.02
Initial weight	1	181.4***	0.06	232.8***	0.08	151.7***	0.05	55.3***	0.02
Overall			0.38		0.33		0.36		0.30

* $P < 0.05$, *** $P < 0.001$

The significance of the differences in least square means for strains and sexes was tested using the Tukey-Kramer method. The test adjusts for multiple comparisons, and thus controls family wise error for unequal sample sizes in experimental groups. In addition, other highly conservative tests in SAS were also used to verify statistical differences between pair-wise groups.

We also analyzed survival rate of the fish from stocking to harvest, using a generalized non-linear model. Survival was considered as binary trait, and the model assumed that the data followed a binomial distribution, with a logit link function ($p^{\wedge} = e^x / (1 + e^x)$). Means of strains for survival were back-transformed from the logit scale to the proportional observations.

RESULTS AND DISCUSSION

Descriptive statistics

Basic statistics for body traits pooled across strains are given in Table 2. The fish reached a harvest weight of 259 g over 134 days of culture from stocking at around 12 g, with average daily weight gain of 1.84 g. The growth rate of these strains was comparable to other red tilapia of various origins reported in the literature under different culture environments in several countries (Hulata *et al.* 1995; Romana-Eguia and Eguia, 1999; Moreira *et al.* 2005; Pongthana *et al.*, 2008 submitted). In the present study, the coefficient of variation in body weight was large (33%), consistent with other studies for *Oreochromis spp* (Pongthana *et al.*, 2008 submitted) and Nile tilapia (Ponzoni *et al.*, 2005; Nguyen *et al.*, 2007). In a review, Gjedrem (1983) also showed a very wide range of coefficient of variation for body weight at various ages in different fish species (13 to 78%).

Table 2. Number of records (N), mean, standard deviation (SD), coefficient of variation (CV, %) for body traits of red tilapia

Traits	Unit	N	Mean	SD	CV (%)	Min	Max
Weight	g	2015	258.6	84.7	32.7	72.0	593.0
Length	cm	2015	18.5	1.9	10.1	11.3	23.5
Depth	cm	2015	3.1	0.5	15.4	1.8	4.4
Width	cm	2015	7.9	1.1	14.4	5.0	11.7

Strain performance

Table 3 shows growth performance of red tilapia strains from Malaysia, Thailand and Taiwan. The Malaysian strain had the fastest growth rate, whereas the lowest performing strain was that from Thailand. The growth performance of the Taiwanese strain was intermediate.

Table 3. Least square means of body traits for each strain of red tilapia

Strains†	Weight	Length	Width	Depth
MY	282.3 ± 4.5 ^a	18.5 ± 0.10 ^a	8.0 ± 0.06 ^a	3.2 ± 0.03 ^a
TL	234.0 ± 2.9 ^b	18.1 ± 0.07 ^b	7.6 ± 0.04 ^b	3.0 ± 0.20 ^b
TW	255.2 ± 3.1 ^c	18.7 ± 0.07 ^{ac}	7.9 ± 0.04 ^{ac}	3.0 ± 0.20 ^c

† MY= Malaysia, TL= Thailand and TW= Taiwan Means sharing superscript letters in a column are not statistically different

These results are in good agreement with those obtained from a separate study carried out in Thailand by Pongthana *et al.* (2008, submitted) on Taiwan and Thai strains of the same origin. In the latter study, the fish were tested in both freshwater cages (0 ppt) and saline water (30 ppt). In the freshwater environment, ranking of the

Malaysia strain was at the top. Note that the Malaysia strain in the study of Pongthana *et al.* (2008 submitted) was of different origin from the one used in our current study. It was introduced to Thailand from a private hatchery in Malaysia. In this study, the 'Malaysia' strain was formed from four different stocks, chosen from strain evaluation experiments previously conducted in Jitra and FFRC. This could be one reason to explain why the Malaysia strain showed significantly better growth performance than those from Taiwan and Thailand in the present study. Regardless of the origin of the strains used in both our study and in that of Pongthana *et al.* (2008 submitted), genetic background and selection history of the three red tilapia strains is not well known. They also do not represent the respective countries. In reality, the fish from Taiwan were imported from a private hatchery to Pathumthani Fisheries Test and Research Center (PFTRC, Thailand) where the stock has been maintained as one of the base materials for the breeding program. We obtained both the Taiwan and Thai strains from PFTRC. The Thai red tilapia, although it was originally developed from the red variant *O. mossambicus* through mass selection, it was subsequently hybridized with various pure and mixed strains of *O. niloticus* to improve its performance. Therefore, the exact genetic composition of all the strains used in the present study is not clearly defined. In general, red tilapia strains cultured in Asian countries comprise a gene pool of two main species: *O. niloticus* and *O. mossambicus*. One exception is red tilapia strains from Philippines that resulted from hybridization of *O. mossambicus* × *O. urolepis hornorum* hybrid females and Japanese *O. niloticus* males (Briggs, 1981). In a comparison experiment of the red tilapia strains from Philippines with those from Taiwan and Thailand, Romana-Eguia and Eguia (1999) reported that the former performed best in saline water, whereas in freshwater environment the fastest growth strain was from Taiwan. A rigorous comparison among studies is impossible due to differences in the origin of the strains, testing environments as well as other management practices. Our results show that there is genetic difference in performance among the strains studied. However, we will further examine the additive and non-additive genetic performance of the strains in a complete diallel cross, which is underway.

Sex effect

Figures 1 and 2 show that males of red tilapia had significantly greater body dimensions than those of females. Statistical differences between the two sexes were highly significant ($P < 0.001$) for all traits. Sexual dimorphism in growth has been reported in many aquatic species such as Nile tilapia (Ponzoni *et al.*, 2005; Nguyen *et al.*, 2007), common carp (Ninh *et al.*, unpublished results), crayfish (Jerry *et al.*, 2005) and giant freshwater prawn (Thanh *et al.*, in preparation). For red tilapia, Pongthana

et al. (2008 submitted) found that between male and female differences in body measurements were of the order of up to 50%. This figure was from 4 to 12% in the present study.

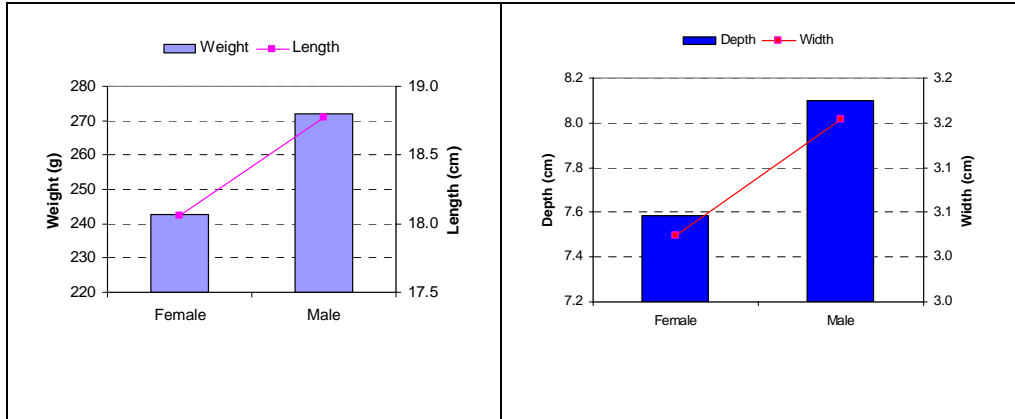


Figure1: least square means of body weight (bar) and standard length (line) for female and male

Figure2: least square means of body depth (bar) and width (line) for female and male

In general, growth differential between sexes is critical for farmed aquaculture species. However, there is very limited scope for selection for sexual dimorphism because the genetic correlations between the expressions of body traits in females and males are almost unity (Nguyen *et al.*, 2007). Hence, means of producing all male tilapia population merit further study. One possibility is to increase male to female ratio by applying thermal treatment to newly hatched fry as observed in Nile tilapia (Baroiller and D'Cotta, 2001). If the technique also worked effectively in red tilapia, there would be possibilities of conducting selective breeding for sensitivity to the treatment. There are also other alternative methods such as manual sexing, hormone sex reversal or YY male technology. However, none of them have been shown to be cost effective and sustainable. From management point of view, minimizing variation in body traits between sexes can be done through improved management practices such as synchronization of fry production, improved nutrition or reduced stocking density.

Strain by sex interaction

The effect of strain by sex was highly significant for all body traits ($P < 0.001$). Overall, females of the Thai strain had significantly lower body weight than either females or males of other groups ($P < 0.01$ to 0.001). Between sex differences in weight, length, depth and width were all significant ($P < 0.05$) in Thai and Taiwan strains. For the Malaysian strain, measurements of body traits were consistently greater in males than in females; however, the differences between the two sexes were not significant ($P > 0.05$, Table 4).

Table 4. Growth performance of female and male for strains of red tilapia

Strain	Sex	Weight	Length	Width	Depth
MY	F	276.0 ± 5.85 ^{abcf}	18.3 ± 0.13	7.8 ± 0.08	3.2 ± 0.04
	M	288.7 ± 4.88 ^b	18.6 ± 0.11	8.2 ± 0.07	3.3 ± 0.03
TL	F	209.2 ± 4.18 ^c	17.5 ± 0.10	7.3 ± 0.06	2.9 ± 0.03
	M	258.9 ± 3.39 ^{df}	18.7 ± 0.08	8.0 ± 0.05	3.1 ± 0.02
TW	F	242.5 ± 4.36 ^e	18.3 ± 0.10	7.7 ± 0.06	3.0 ± 0.03
	M	268.0 ± 3.81 ^f	19.1 ± 0.09	8.1 ± 0.05	3.1 ± 0.02

Means sharing superscript letters in a column are not statistically different ($P > 0.05$). Pair-wise comparisons for length, width and depth are consistent with those of body weight

Despite the strain by sex interaction was statistically significant, it accounted for only very small proportion of total variation in the model (about 3% across traits). Further, the magnitude of differences between sexes in terms of phenotypic standard deviation units (σ_p) for body traits was generally small, especially between the Malaysian and Taiwan strains (e.g. 0.50 vs. 0.39 σ_p for body width). This is indicating that strain by sex interaction is likely due to scaling effect rather than re-ranking of strain across genders. A graphical presentation of the least square means for body traits in Figure 3 also demonstrates that the connection lines of means between strains are almost parallel. For all body traits (weight, length, width and depth), the interaction effects (I) were smaller than the strain (G) and sex (S) effects. Using body weight as an example, the average effect of Malaysia and Taiwan strains across sexes was 268.8 g, and the average effect of sexes over the two strains was 201.2 g. These figures for both the strain and sex were remarkably greater than the interaction effect (only 12.8 g), which was calculated as $(MY_M + TW_F) - (MY_F + TW_M)$ where M and F refer to male and female of Malaysia (MY) and Taiwan (TW) strains. In most such cases, the strain by sex interaction was not important. The effect of G×S interaction is significant when $I > S > G$ or $I > G > S$ (reviewed by Mathur, 2002). This pattern is not observed for any trait in the present study. Given with the current data structure, our interpretation of strain by sex interaction based on conventional method of analysis of variance. However, a rigorous evaluation of strain or genotype by sex interaction would require an estimation of genetic correlations for homologous traits between sexes when sufficient data can be accumulated in a full pedigree with sufficient depth (see Nguyen *et al.*, 2007). This type of analysis can be performed using REML (restricted maximum likelihood) procedures under framework of mixed model methodology.

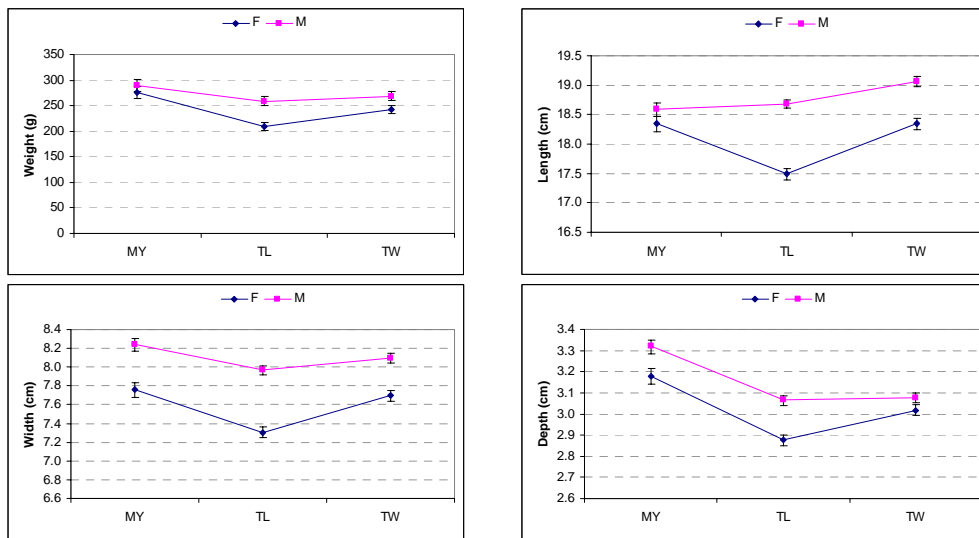


Figure 3: Least square means of body traits for female (F) and male (M) of Malaysia (MY), Thai (TL) and Taiwan (TW) strains. Standard errors of weight time two.

Survival

Survival rate of three red tilapia strains from stocking to harvest is presented in Table 5. In contrast to the results for body traits, survival rate of the Malaysian strains was lowest and statistically different ($P < 0.001$) from that of the Thai and Taiwan strains. However, the difference in survival rate between Thai and Taiwan strains was not statistically significant.

Table 5. Survival rate of red tilapia strains from stocking to harvesting

Scale	MY	TL	TW	Significant probability
Logit value	0.68 ± 0.06^a	1.29 ± 0.07^b	1.43 ± 0.08^b	$P < 0.001$
Observed scale (%)	66.4	78.5	80.6	

Standard errors were not estimated on observed scale

The exact reasons for the lower survival of Malaysia than other strains are not known. However, we suspect that this may be attributed to a higher proportion of small fish at stocking of the Malaysia relative to the other strains. Due to long interval between spawning batches, there were insufficient fingerlings of similar (uniform) size for the experiment. The small fish from the Malaysian strain may have been disadvantaged from food competition in early stage of grow-out, and they could be more vulnerable to environmental conditions and thus having a higher risk of deaths. Furthermore, the Malaysia strain was found to be less aggressive compared to Thailand and Taiwan strain; they could also be attacked by others or predators. The variation in initial weights of the Thai and Taiwan strain is generally less pronounced because the fry were sampled from mass spawning production, and reared all together in the same hapas. By contrast, single pair mating was carried out in the Malaysian stock, and the fish spawned asynchronously. After hatching individual family

was also kept in separate hapas in pond until the fry reached a suitable size for physical tagging. Although age and initial weight differences between strains were included in the statistical models, there are also other factors contributing to variation in survival rate in this experiment.

The strong influence of environmental factors on survival rate is indicated by the low level of heritability for this trait in several species (around 0.1). In the nucleus, survival should be included in breeding objectives because the trait determines number of fish available for market, and thus profit of fish farming. Under practical production systems, improvement of survival rate can be achieved by modification of environmental factors, such as better management, feeding, diet or water quality.

For survival, data recording and analysis methods for this trait reported in the literature have raised concerns. In almost all studies where full pedigree or measurement of individual fish is not available, survival is often expressed as average percentage of the difference between stocking and harvest fish. In this way, there is a very limited number of observations per treatment group. Thus, results from analysis of variance (ANOVA) are often biased, leading to misinterpretation of statistical differences between the treatments. The classical method of ANOVA is mostly used after the data is normalized using (arcsine)-square root transformation. In the present study, we recorded survival on individual fish, and modeled survival rate as a binary trait with a logit link function, assuming that the data followed binominal distribution. Note that the retention of PIT tags in this study was almost 100%. Tag loss occurred mainly after a few days of tagging, but the 'lost tag' fish were replaced by siblings of the same strain before stocking. Hence, survival rate is not confounded by tag loss in the present study. In summary, since survival rate differed among strains, there may be a within strain genetic component in this trait that could be taken into account in breeding programs for red tilapia.

CONCLUSION

Strain evaluation experiments generally involve several steps from choice of strains, population sampling, preparation of testing environments, determination of sample sizes, implementation of the experiment, collection of data, statistical analysis and interpretation of the results. In addition, we strictly followed all steps as suggested in the manual for "Strain Comparisons in Aquaculture Species" by Nguyen and Ponzoni (in revision), but some shortcomings could not be avoided in this experiment. However, most of possible systematic effects associated with the observed traits were generally accounted for in statistical models. Our results are consistent with those obtained from an independent study by Pongthana *et al.* (2008 submitted), indicating that there are genetic differences in growth performance and

survival among red tilapia strains from Malaysia, Taiwan and Thailand. The best performing fish from each strain are being crossed following a full diallel design to further examine their additive and non-additive performance before forming the base population for future genetic selection. Due to the specific characteristics of the species, we also consider different selection strategies to simultaneously improve performance and coloration of the fish in the planned breeding program. Other traits of economic importance will also be included in the breeding objective as the program unfolds. The ultimate aim of this project is to develop a fast growing strain of red tilapia with uniform coloration, and having good adaptation to a wide range of farming production systems in Malaysia.

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