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Additive genetic and heterotic effects in a 4 × 4 complete diallel cross-population of Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758) reared in different water temperature environments in Northern Vietnam

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Abstract

This study aims to estimate the strain additive genetic and heterotic effects on growth and survival in a 4 × 4 complete diallel cross-population of Nile tilapia. Mass spawning was practised in replicate hapas to simultaneously produce progeny of all crosses for performance testing in three environments (in ponds at 20–30°C, in tanks at 15–20°C and in tanks at 20–25°C). A total of 6735 individually tagged fish were tested over a grow-out period of 278 days. Statistical analyses were carried out on 5097 body trait records available at harvest. Across the test environments, the NOVIT4 strain exhibited the highest additive genetic values for both growth and survival (19% and 33% above the pure strain mean respectively). The heterosis effect was low and not different from zero for both traits. The ranking of strains with respect to their additive genetic values generally did not change between tank environments (15–20 vs. 20–25°C). The correlations of the additive genetic performance between tank environments were also high (0.84), suggesting that strain by water temperature interaction was likely not biologically important. By contrast, the differences in both performance and survival between pond and tank environments were statistically significant, indicating that this effect should be accounted for in future breeding programmes. The large additive genetic effect among strains coupled with the non-significant heterotic effects in our study suggest that future breeding programme in this population of Nile tilapia should be based on a wise choice of strain or by exploiting the additive genetic variation through selective breeding.

Keywords: additive genetic, cold tolerance, diallel cross, heterotic effect, Nile tilapia, *Oreochromis niloticus*

Introduction

Culture of tilapias in Northern Vietnam experiences unfavourable low water temperatures in ponds during winter from 10–18°C for about 4 months from December to March. As tilapias (family Cichlidae) are of tropical and subtropical origin and have optimal growth between 25 and 32°C, the low temperatures during winter in Northern Vietnam have led to poor performance and high mortality of both adult and juvenile fish and consequently low economic efficiency (Dan & Little 2000). Successful over-wintering in ponds have been practised using heated facilities, geothermal water and greenhouses insulated with plastic covers (Dan & Little 2000). Crab, Kochva, Verstraete and Avnimelech (2009) also showed the effectiveness of bio-flocs technology in maintaining water quality in over-wintering ponds for tilapia. Besides environmental manipulation, dietary supplements such as polyunsaturated fatty

acids, vitamin E and carnitine have been shown to promote cold tolerance in fish (Harpaz, Becker & Blum 1999; Snyder & Murray 2009).

In addition to improving the environment, management and nutrition, genetics is also seen as a way to improve cold tolerance capacity of the animals. Several previous studies reported significant variation in cold tolerance among tilapia species with *O. aureus* being the most tolerant, *O. mossambicus* the least and *O. noliticus* exhibiting intermediate tolerance (Behrends, Kingsley & Bulls 1990). There is also additive genetic component for cold tolerance (Tave, Smitherman & Jayaprakas 1989; Sifa, Chenhong, Dey, Gagalac & Dunham 2002; Armas-Rosales 2006), although others (Cnaani, Gall & Hulata 2000) have shown a large component of the trait's variance was a result of dominance effect. Within a strain, the heritability for cold tolerance is generally low to moderate across species (Charo-Karisa, Rezk, Bovenhuis & Komen 2005; Thodesen, Rye, Wang, Li, Bentsen & Gjedrem 2013), suggesting the possibility for improvement of this trait through selective breeding.

As a first step to gain understanding of strain and heterosis effects on growth and survival under different water temperature environments, we conducted a full 4 × 4 diallel cross to evaluate genetic components of potential tilapia strains available in Vietnam. The diallel cross-approach was taken because it allows to separate genetics from non-genetic components and to estimate reciprocal (maternal) effects as well as to provide a basic information regarding strain by environment interaction. This approach has been successfully practiced to enlarge genetic variability in foundation populations to ensure long-term response to selection in several farmed aquaculture species, including Atlantic salmon *Salmo salar* (Gjedrem, Gjøen & Gjerde 1991), GIFT tilapia (Genetically Improved Farmed Tilapia, *O. niloticus*) (Bentsen, Eknath, Palada-de Vera, Danting, Bolivar, Reyes, Dionisio, Longalong, Circa, Tayamen & Gjerde 1998; Eknath, Bentsen, Ponzoni, Rye, Nguyen, Thodesen & Gjerde 2007; Nguyen, Ponzoni, Abu-Bakar, Hamzah, Khaw & Yee 2010), red tilapia *Oreochromis* spp. (Pongthana, Nguyen & Ponzoni 2010), common carp *Cyprinus carpio* (Ninh, Ponzoni, Nguyen, Woolliams, Taggart, McAndrew & Penman 2013), Indian 'Jayanti' rohu carp *Labeo rohita* (Gjerde, Reddy, Mahapatra, Saha, Jana, Meher, Sahoo, Lenka, Govindassamy & Rye 2002) and Giant freshwater prawn *Macrobrachium rosenbergii* (Thanh, Nguyen, Ponzoni, Vu, Barnes & Mather 2010; Hung, Vu, Nguyen, Ponzoni, Hurwood & Mather 2013).

In the present study, we report estimates of the strain additive genetic and heterotic effects for body weight and survival at harvest of four Nile tilapia strains (*O. niloticus*) and their crosses reared over a grow-out period of 9 months in different freshwater temperature environments in Northern Vietnam. The ultimate aim of the study is to obtain basic information to establish alternative breeding strategies for a future genetic improvement programme of this species (*O. niloticus*).

Material and methods

Origins of strains

This study involved four strains of Nile tilapia, originating from Taiwan (D), Israel (I), Vietnam (N) and Thailand (T). The Taiwanese strain of Nile tilapia was introduced from Taiwan to Southern part of Vietnam in 1973 and was then transferred to culture in the North. The breeding stock has been kept in the National Life Gene Bank since 1978 at National Broodstock Center for freshwater species under the Research Institute for Aquaculture No. 1 (RIA1). The Israeli strain of Nile tilapia was introduced from Israel to RIA1 in 2006 (Khanh 2010). The strain from Vietnam is named as NOVIT4 which is the GIFT- derivative selected over ten generations for high growth under freshwater environment at RIA1 (Luan, Olesen, Ødegård, Kolstad & Dan 2008). The Thailand strain of Nile Tilapia was introduced to Research Institute for Aquaculture No.1 (RIA.1), Vietnam in 1994 from Asian Institute of Technology (AIT), Thailand. All stocks were maintained and reared under the same culture conditions at RIA1 before the experiment. In April 2011, a complete diallel cross involving the four strains was carried out using brooders randomly taken from each strain (the average body weight of brooders were 400 g for females and 500 g for males). Mass spawning was practiced to simultaneously produce 16 crosses (60 male and 60 female brooders per cross).

Fry production and rearing

The mass spawning of the 16 crosses were conducted in separate breeding hapas (5 × 4 × 1 m)

over 3 months (from April to June 2011) in RIA1 (latitude 21°N, longitude 105°E, 15 km north of Hanoi). Before mating, the female and male breeders were conditioned in separate hapas (20 × 5 × 1.5 m) for 1 month. The female was transferred to the breeding hapa before the male was introduced. A total number of 48 breeding hapas were installed in an earthen pond (40 × 30 × 1.5 m). In each hapa, 20 females were stocked with 20 males and three replicate mating hapas were used for each cross. All hapas were inspected daily for the presence of swim-up fry. The swim-up fry were collected from each hapa and then transferred to 3 × 2 × 1 m rearing hapas at a stocking density of 5000 fry per hapa, one hapa for each cross per collection. The collection date of swim-up fry was recorded for each cross. During the first 5 weeks of nursing in hapas, the fry were fed four times per day, a commercial powder feed (Cargill 7424) containing 40% crude protein at the rate of 15% of their body weight. After the initial rearing stage, fry/fingerlings were transferred to B-net hapas (~6 mm mesh size) at stocking density of 500 fingerlings per hapa (2.5 × 2 × 1 m) for further nursing for another 75 days, and they were fed a commercial pellet (30% protein). After this nursing period in B-net hapas, a random sample of 500 fingerlings per cross was individually tagged, using Passive Integrated Transponders (PIT). Average body weight of the fingerlings at tagging was 15 g and there was no significant statistical difference in stocking weight among crosses. After tagging, the fingerlings were conditioned for 10 days in hapas. During this period there were some mortality losses. A total of 6735 tagged fingerlings remained and were then communally stocked in three testing environments.

Grow-out testing environments and harvest

Ten days after tagging, the fish were stocked at RIA1 in three different water temperature environments: 1) in a pond of 50 × 40 × 1.5 m at 20–30°C, 2) in two tanks of 5 × 5 × 1.2 m at 15–20°C and 3) in two tanks of 5 × 5 × 1.2 m at 20–25°C. The stocking density of fish in the tested tank environments (30 fish per cross per tank) and in the tested pond of 2000 m^2 (300 fish per cross) was 25.6 per cubic metre and 2.5 fish per m^2 of surface water respectively. The fish were fed twice a day by a commercial pellet feed having a dietary protein level of 22%, at the rate of 3–4% of their body weight, adjusted corresponding to the growth potential of the fish in different culture periods. Sampling was made monthly to measure the fish body mass in each environment. In all environments, water quality parameters such as temperature, pH and dissolved oxygen level were monitored daily; other parameters (alkalinity and total ammonia level) were measured once a week. Ambient temperature was recorded in the pond testing environment, whereas temperature in the tested tanks was maintained by an auto set temperature control system.

Following a grow-out period (232–301 days in tanks and 260–329 days in pond), all fish were harvested and immediately transferred to large hapas for 2 days of conditioning without feeding before the individual identification, body measurements and sex were recorded. Body measurements included live weight, total length, body height and body depth. The correlations among body traits, which measured above, were very high (close to one); hence, only live weight is reported in this study. The number of fish recorded in each cross and environment at stocking and harvest was used to calculate survival rate. In the present study, survival rate included both dead and tag lost fish. The tag loss rate was about 9.8% across the grow-out testing environments. Basic statistics for live weight and survival rates at harvest in each environment are presented in Table 1.

Statistical analysis

Statistical analyses were performed on 6735 performance and survival data records collected from

Table 1 Descriptive statistics for body weight and survival of a 4 × 4 diallel cross-population of Nile tilapia in three testing environments

Traits	Environments	*N*	Mean	SD	CV
Weight, g	P	3534	247.3	123.9	50.1
	T1	824	123.8	64.9	52.5
	T2	739	122.5	58.2	47.5
	Overall	5097	217.8	124.4	57.1
Survival, %	P	4800	70	0.44	59.9
	T1	969	80	0.43	55.8
	T2	966	90	0.35	41.5
	Overall	6735	80	0.43	56.6

N = number of fish with records at harvest; SD = standard deviation; CV (%) = coefficient of variation; P = pond (20–30°C), T1 = tank (15–20°C); T2 = tank (20–25°C).

a 4 × 4 complete diallel cross-population of Nile tilapia at RIA1. Preliminary analyses using a general linear model (GLM) procedure in SAS (SAS Software, 1997) were performed to test the statistical significance of systematic effects associated with weight and survival. The fixed effects included cross-combinations, environment, sex and their two way interactions and a linear regression on age at harvest. Initial stocking weight did not differ among crosses and when it was fitted in the model, the partial R^2 value remained unchanged. Hence, stocking weight was not included from the final models to analyse traits studied. The final model is written in mathematical expression as the followings:

$$y_{ilklm} = \mu + G_i + E_j + S_k + (G \times E)_{ij} + (G \times S)_{ik} + (E \times S)_{jk} + b_1 A_l + e_{ilklm} \quad (1)$$

where y_{ilklm} is the measurements of a body trait at harvest, μ is a constant, G_i is the fixed effect of cross-combinations (i = 16, four pure strains and 12 crosses), E is the test environments (j = 1, 2, 3 corresponding to three culture environments in pond at 20–30°C, in tank at 15–20°C and in tank at 20–25°), S is sex (k = female and male), $G \times E$ is the interaction between genotypes and environments, $G \times S$ the genotype by sex interaction and $E \times S$ the environment by sex interaction, A_l is the age at harvest, and e_{ilklm} is the random residual term.

The effect of genotypes given in Model 1 was partitioned into terms of direct additive, non-additive and reciprocal effects. The mathematical expression of the model is as follows:

$$y_{ij} = \mu + E_j + S_k + (E \times S)_{jk} + b_1 A_l + \sum \alpha_i a_i + \sum \alpha_{ij} h_{ij} + \sum \beta_i r_i + e_{ijk} \quad (2)$$

where E and S are the fixed effects of environment and sex and covariate of age as described above, α_i is the proportion of genes contributed by the nth individual originating from the i^{th} strain (α_i = 0.0, 0.5 or 1.0 and $\Sigma\alpha_i$ = 1.0); a_i is the additive genetic effect of genes originating from the ith strain; α_{ij} is the coefficient of the total heterosis effect for the cross between the ith and jth strains (α_{ij} = 0.0 or 1.0; $i \neq j$ and $ij \neq ji$ and $\Sigma\alpha_{ij}$ = 1.0); h_{ij} is the total heterosis effect for the cross between the i^{th} and j^{th} strains ($i \neq j$ and $ij \neq ji$); βi is the coefficient of the general reciprocal effect for the ith strain (β_i = 0 for purebreds and −0.5 for male strain and 0.5 for female strain, for the crossbreds and $\Sigma\beta_i$ = 1.0); r_i is the general reciprocal effect of the ith strain and e_{ijk} is the random residual error for the l^{th} individual.

The significance of the additive, heterosis and reciprocal effects was assessed using the partial F test by removing each term from the full model at a time (Myers & Well 2003). The additive, heterosis and reciprocal effects were estimated as regression coefficients with one degree of freedom. The additive genetic effects were restricted to $\sum a_i = 0$. Due to the limited number of sires and dams per cross, the coefficients of the general reciprocal effects set in the present study assume that the additive genetic effects of a given strain are the same regardless of gender of parental breeders (Gjerde *et al.* 2002). The inclusion of the reciprocal effects aimed to increase accuracy of the additive genetic and heterotic estimates. Total heterosis for a cross between two strains was partitioned as $h_{ij} = \bar{h} + h_i + h_j + s_{ij}$, where $\bar{h}$ is the average heterosis effect for all strains involved in the diallel cross, h_i and h_j are the general heterosis effects for the i^{th} and j^{th} stock, respectively, and s_{ij} is the specific heterosis effect of strains.

All the analyses were carried out using SAS. The observed body weight was weighted by the reciprocal of the variance within environment, using the WEIGHT option in PROC GLM of the SAS package. The approach is as used by Gjerde *et al.* (2002) and Maluwa and Gjerde (2006). We found that this method resulted in the smallest standard errors of the estimates relative to other transformations such as weighing each record by the inverse of the standard deviation or by the inverse of the variance (Thanh *et al.* 2010).

Results

Basic statistics

Descriptive statistics for harvest body weight and survival of Nile tilapia from tagging to harvest in three testing environments are shown in Table 1. The mean of body weight from the pond was two times greater than that in the two tank environments (247.3 g in pond compared with 123.8 and 122.5 g in T1 and T2 environments, correspondingly). This is likely due to the

differences in stocking density, harvesting age, water temperature between ponds and tank environments as well as due to other management and environmental factors. Survival rate from stocking to harvest were relatively high across testing environments, ranging from 70% to 90%. The dead and tag lost fish in this study was approximately 23%. The coefficients of variation were considerably greater for harvest weight and survival than for standard length.

Fixed effects

Table 2 presents the statistic analysis of fixed effects on body weight and survival. The effects of environment, cross-combinations, sex and their interactions were highly significant ($P < 0.0001$) for both traits studied (body weight and survival). The regression of age at harvest on body weight was generally linear ($P < 0.0001$).

The growth performance and survival of the four pure strains of Nile tilapia and their reciprocal crosses in the three different water temperature environments are shown in Figures 1 and 2. Among the pure strains, the NOVIT4, which is the GIFT- derivative selected over ten generations at RIA1, had the greatest growth rate in the pond environment. By contrast, the strain from Israel ranked top in the tank environments. In both tank environments, the NOVIT4 also had high ranking (second). Across the three testing environments, the strain from AIT Thailand exhibited the poorest growth rate. With regard to strain combinations, crosses involving NOVIT4 females with Taiwanese males (TD, ID, TN and IN) exhibited better growth than other crosses in the pond environment. For survival rate, the Israeli strain showed the highest viability in T2 environment (20–25°C in tank), whereas the NOVIT4 strain ranked highest in the pond (20–30°C). The survival rate of the cross between Thai males and Taiwanese females was lowest. Across the three environments, the pure strain of NOVIT4 showed the highest survival rate.

Strain additive genetic effect

The estimates of strain additive genetic effects on body weight and survival are presented in Table 3. For body weight, the NOVIT4 strain showed the highest additive genetic value in pond and T2 environment (the water temperature above 20°C). By contrast in the T1 environment (15 to 20°C), the ranking of pure strains in the descending order of the additive genetic performance was Israel, NOVIT4, Taiwan and Thailand strain. Across test environments, the top ranked strain was NOVIT4, followed by the stock from Taiwan (32.9%, 19.8% above the overall mean, respectively), whereas the additive genetic performance of the strain from Israel was poorest (35.9% below the overall mean). Among the crossbreds, the one with Taiwanese males and NOVIT4 females had top ranking in both the pond and T2 (water temperature >20°C) as well as across the three environments. A crossbred between NOVIT4 males and Thailand females had additive genetic value equal to the combination between the Taiwanese and NOVIT4 strains.

Table 2 Significance of fixed effects on weight and survival

	Weight			Survival		
Effects	Degree of freedom	*F*-value	Probability	Degree of freedom	*F*-value	Probability
E	3	78.6	<0.0001	3	65.5	<0.0001
G	15	28.9	<0.0001	15	854.9	<0.0001
G*E	45	10.2	<0.0001	45	333.8	<0.0001
S	1	53.9	<0.0001			
E*S	3	74.0	<0.0001			
G*S	15	10.7	<0.0001			
Age (S, E, C)	32	654.4	<0.0001			
R^2	0.66					

E = environments (pond, tank 1 and tank 2); G = genotype (or cross-combination); S = sex. Sex and age of dead fish were not known; hence they are not included in the model for survival.

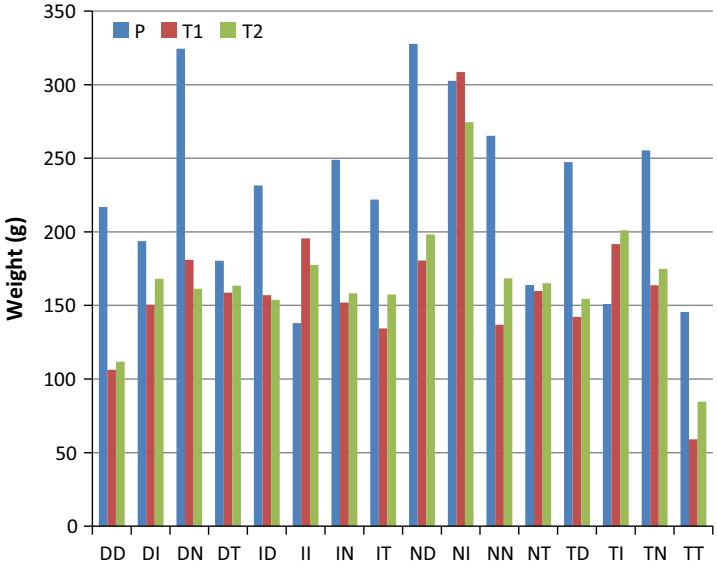

Figure 1 Least squares means for body weight of 16 crosses (males × females) of Nile tilapia in three different testing environments (P = pond at 20–30°C; T1 = tank at 15–20°C; T2 = tank at 20–25°C. Strain designation: D = Taiwan; I = Israel; N = NOVIT4 (GIFT-derived strain by RIA1) and; T = Thailand. Crosses = males × females.

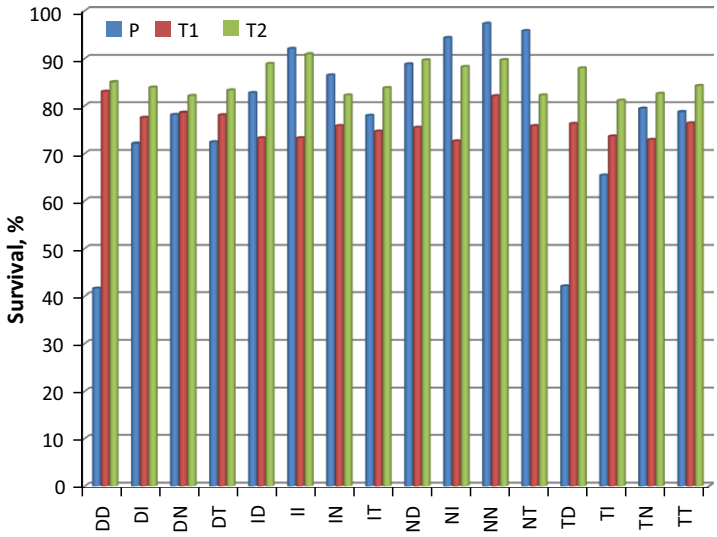

Figure 2 Survival rate of 16 crosses (males × females) of Nile tilapia in three different testing environments (P = pond at 20–30°C; T1 = tank at 15–20°C; T2 = tank at 20–25°C). Strain designation: D = Taiwan; I = Israel; N = NOVIT4 (GIFT-derived strain by RIA1) and T = Thailand. Crosses = males × females.

With regard to the effects of strain additive genetics on survival, among pure strains, the NOVIT4 strain had the highest additive genetic value in the pond (26.8% above the mean), but the highest ranking strains in tanks (T1 15–20°C) and T2 (20–25°C) were those from Taiwan and Israel (5.5% and 3.9% above the mean respectively). Overall, the NOVIT4 strain was ranked top (18.6% above the mean) across test environments, whereas the Taiwanese strain was lowest (33.0% below the overall mean). Among the cross-combinations, the ranking of additive genetic value generally varied with testing environments. Across the three environments, the highest additive genetic value (11.1% above the pure strain mean) was obtained in the cross between Israeli males and NOVIT4 females, whereas the additive genetic performance of the cross between Taiwanese males and Thailand females was lowest (19.0% below the pure strain mean).

Heterosis effects

Total and average heterosis effects for body weight and survival at harvest are given in Table 4. Heterosis components for body weight varied with

Table 3 Estimates of additive genetic effects for harvest weight and survival

	Weight								Survival							
	P		T1		T2		Overall		P		T1		T2		Overall	
	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%
Mean	244.8		117.9		126.7		244.6		73.3		78.7		87.5		78.6	
Pure strain																
D	42.1	17.2	11.2^{ns}	9.5	11.2^{ns}	8.9	48.4	19.8	−34.1	−46.5	4.4^{ns}	5.5	−2.5^{ns}	−2.8	−25.9	−33.0
I	−73.0	−29.8	18.4^{ns}	15.6	−15.2^{ns}	−12.0	−87.7	−35.9	12.9	17.6	−5.5^{ns}	−7.0	3.4^{ns}	3.9	9.7	12.4
N	71.5	29.2	13.7	11.6	30.9	24.4	80.5	32.9	19.7	26.8	3.4^{ns}	4.3	2.2^{ns}	2.5	14.6	18.6
T	−40.6	−16.6	−43.2^{ns}	−36.7	−27.0^{ns}	−21.3	−41.2	−16.8	1.5ns	2.0	−2.3^{ns}	−2.9	−3.2^{ns}	−3.6	1.6^{ns}	2.0
Crosses																
DI	−15.4	−6.3	14.8^{ns}	12.5	−2.0^{ns}	−1.6	−19.7	−8.0	−10.6	−14.4	−0.6^{ns}	−0.7	0.5^{ns}	0.5	0.3	0.4
DN	56.8	23.2	12.4^{ns}	10.5	21.1^{ns}	16.6	64.4	26.3	−7.2	−9.8	3.9^{ns}	4.9	−0.1^{ns}	−0.1	4.6	5.9
DT	0.7^{ns}	0.3	−16.0^{ns}	−13.6	−7.9^{ns}	−6.2	3.6^{ns}	1.5	−16.3	−22.2	1.0^{ns}	1.3	−2.8^{ns}	−3.2	−15.0	−19.0
IN	−0.7^{ns}	−0.3	16.0^{ns}	13.6	7.9^{ns}	6.2	−3.6^{ns}	−1.5	16.3	22.2	−1.0^{ns}	−1.3	2.8^{ns}	3.2	8.7	11.1
IT	−56.8	−23.2	−12.4^{ns}	−10.5	−21.1	−16.6	−19.7	−8.0	7.2	9.8	−3.9^{ns}	−4.9	0.1^{ns}	0.1	−4.4	−5.6
NT	15.4	6.3	−14.8^{ns}	−12.5	2.0^{ns}	1.6	64.4	26.3	10.6	14.4	0.6^{ns}	0.7	−0.5^{ns}	−0.5	6.2	7.9

P = pond (20–30°C); T1 = tank (15–20°C); T2 = tank (20–25°C); D = Taiwan; I = Israel; N = NOVIT4 (GIFT-derived strain by RIA1); T = Thailand; Crosses = males × females; ns = non-significant ($P > 0.05$); Est. = estimate.

Table 4 Estimates of heterosis components for harvest weight and survival

	Weight								Survival							
	P		T1		T2		Overall		P		T1		T2		Overall	
	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%
Mean	244.8		117.9		126.7		244.6		73.3		78.7		87.5		78.6	
General heterosis																
D	−2.5^{ns}	−1.0	−21.4^{ns}	−18.2	−24.3	−19.2	−3.6^{ns}	−1.5	14.0	19.2	−3.6^{ns}	−4.6	−0.7^{ns}	−0.8	5.3	6.8
I	2.7^{ns}	1.1	−11.0^{ns}	−9.4	−11.8^{ns}	−9.3	3.8^{ns}	1.5	2.3^{ns}	3.1	−2.3^{ns}	−2.9	−3.9^{ns}	−4.5	−1.7^{ns}	−2.2
N	17.0	7.0	5.7^{ns}	4.8	−16.0^{ns}	−12.7	16.3	6.7	10.9	14.8	−4.6^{ns}	−5.9	−3.7^{ns}	−4.3	1.7^{ns}	2.1
T	−35.2	−14.4	−5.8^{ns}	−4.9	−16.7^{ns}	−13.1	−40.2	−16.4	−1.8^{ns}	−2.5	−2.8^{ns}	−3.5	−2.8^{ns}	−3.2	−4.9	−6.3
Total heterosis																
DI	−24.3	−9.9	−39.7	−33.7	−30.2	−23.8	−25.8	−10.5	4.1	5.6	−3.3	−4.2	−1.1	−1.2	0.3	0.4
DN	110.8	45.3	12.2	10.3	−4.0	−3.1	118.9	48.6	20.9	28.5	−1.7	−2.1	−1.6	−1.8	4.6	5.9
DT	−51.9	−21.2	−25.6	−21.7	−27.5	−21.7	−55.6	−22.7	−16.9	−23.1	−1.5	−1.9	−1.8	−2.1	−15.0	−19.0
IN	32.7	13.4	39.3	33.3	8.0	6.3	33.1	13.5	17.0	23.2	−4.4	−5.6	−2.4	−2.7	8.7	11.1
IT	−73.4	−30.0	−14.4	−12.2	−28.2	−22.3	−83.7	−34.2	−1.4	−1.9	−4.6	−5.9	−4.8	−5.5	−4.4	−5.6
NT	−20.9	−8.5	−20.8	−17.6	−21.3	−16.8	−22.4	−9.2	14.4	19.6	−4.4	−5.6	−5.0	−5.7	6.2	7.9
Average	−4.5	−1.8	−8.2	−6.9	−17.2	−13.6	−5.9	−2.4	6.3	8.6	−3.3	−4.2	−2.8	−3.2	0.1	0.1

P = pond (20–30°C); T1 = tank (15–20°C); T2 = tank (20–25°C); D = Taiwan; I = Israel; N = NOVIT4 (GIFT-derived strain by RIA1); T = Thailand; Crosses = males × females; ns = non-significant ($P > 0.05$); Est. = estimate.

testing environments. The general heterosis value in the three environments was mostly negative, except for the Israeli and NOVIT4 strains that showed opposite sign in pond and T2 environment. Overall, ranking of the pure strains for their general heterosis effect was highest for the NOVIT4 strain and lowest for the Thailand strain. The total heterosis effects on live weight were also mostly negative. The average heterosis for body weight in each testing environment and across the three environments ranged from −13.6% to −1.8%.

With regard to heterosis components for survival, the general heterosis effects were mostly negative except for Taiwanese, Israeli and NOVIT4 strains in pond environments. A majority of crosses exhibited negative heterosis, except for the cross involving the NOVIT4 strain in the pond. The combination between Israeli and NOVIT4 strains showed highest total hetorosis across test environments (11.1% above the pure strain mean), whereas the combination of Taiwanese and Thailand strains was lowest (−19.0% below the pure strain mean).

Maternal (reciprocal) effects

Overall, the estimates of maternal (reciprocal) effects for harvest weight were positive for the NOVIT4 and Taiwanese strains but negative for the Israeli and Thai strains (Table 5). However, the ranking of strains for their maternal effect changed with the testing environments. In tank culture, the Israeli strain showed highest ranking among the pure strains, whereas in the pond environment the Taiwanese strain had highest maternal value. The maternal effects for survival were generally small and not significant, except for the Thai strain that had positive estimate (14.5% above the pure strain mean).

Discussion

The principal aim of this study was to estimate genetic and non-genetic (heterotic) effects on growth and survival of Nile tilapias under three different water temperature pond and tank environments. The estimates obtained in the present study provided basic information to establish alternative breeding strategies for simultaneous improvement of both growth performance and survival rate in this population in the temperate climate of Northern Vietnam where the annual ambient temperature in winter (December to March) varies from 10 to 20°C.

Our results indicated that strains of Nile tilapia differed greatly with regard to their additive genetic performance and survival in all testing environments. Overall, the NOVIT4 strain had the highest level of additive genetic performance, whereas growth of the strains from AIT Thailand and Israel was below the pure strain mean. The NOVIT4 strain also ranked top with regard to the additive genetic value for survival rate, followed by the stock from Israel. The ranking of the pure strains descending in the order of the additive genetic value for survival was NOVIT4, Israel, Taiwan and Thailand. The significant differences in additive genetic performance and survival among strains involved in the present complete diallel cross could be due to their genetic background, selection history and stock maintenance. The NOVIT4 was selected over seven generations at RIA1, since its introduction from Philippines (the GIFT Foundation) to North Vietnam in 1998. Both stocks from AIT Thailand and Israel were imported to Vietnam in 1994 and 2006 respectively, but they were not selected and only maintained for production at RIA1. The original population size of these stocks was small and genetic drift was inevitable therefore resulting in loss of genetic diversity and performance.

The non-additive genetic component, i.e. the average level of heterosis estimated in the present population was negative and low for both harvest weight and survival rate in individual testing environments or across the three environments. This is in agreement with the estimates in the GIFT (Genetically Improved Farmed Tilapia) strain (Bentsen *et al.* 1998), red tilapia (Pongthana *et al.* 2010), rohu carp (Gjerde *et al.* 2002) and Giant freshwater prawn *Macrobrachium rosenbergii* (Thanh *et al.* 2010; Pillai, Mahapatra, Ponzoni, Sahoo, Lalrinsanga, Nguyen, Mohanty, Sahu, Vijaykumar, Sahu, Khaw, Patra, Patnaik & Rath 2011). Our estimates are, however, considerably lower than those reported for other species such as channel catfish (Argue, Liu & Dunham 2003), common carp (Bakos & Gorda 1995), *O. shiranus* tilapia (Maluwa & Gjerde 2006). Strictly speaking, comparison among studies is not rigorous because the expression of heterosis depends on populations and population (strain) combinations. Heterosis is mainly explained by dominance effects. Generally,

Table 5 Estimates of maternal (reciprocal) effects for harvest weight and survival

	Weight								Survival							
	P		T1		T2		Overall		P		T1		T2		Overall	
	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%	Est.	%
Mean	244.8		117.9		126.7		244.6		73.3		78.7		87.5		78.6	
Pure strain																
D	11.5	4.7	−17.1^{ns}	−14.5	−0.4	−0.3	20.3	8.3	−8.3	−11.3	−2.3^{ns}	−2.9	4.3^{ns}	4.9	−3.2^{ns}	−4.1
I	−8.6^{ns}	−3.5	53.1	45.0	28.1	22.1	−17.2	−7.0	−3.7^{ns}	−5.0	0.0^{ns}	0.0	−0.5^{ns}	−0.5	−2.5^{ns}	−3.2
N	10.3^{ns}	4.2	−29.4	−24.9	−30.7	−24.2	11.6	4.7	−3.3^{ns}	−4.5	0.8^{ns}	1.0	−3.2^{ns}	−3.6	−5.8	−7.4
T	−13.1	−5.4	−6.6^{ns}	−5.6	3.1	2.4	−14.7	−6.0	15.3	20.9	1.5^{ns}	1.9	−0.6^{ns}	−0.7	11.6	14.7
Reciprocal crosses																
DI	1.4^{ns}	0.6	18.0^{ns}	15.3	13.8^{ns}	10.9	1.5	0.6	−6.0	−8.2	−1.1^{ns}	−1.4	1.9^{ns}	2.2	−2.9	−3.7
DN	10.9	4.4	−23.2	−19.7	−15.6	−12.3	15.9	6.5	−5.8	−7.9	−0.8^{ns}	−1.0	0.5^{ns}	0.6	−4.5	−5.8
DT	−0.8^{ns}	−0.3	−11.9^{ns}	−10.1	1.3^{ns}	1.0	2.8	1.1	3.5	4.8	−0.4^{ns}	−0.5	1.8^{ns}	2.1	4.2	5.3
IN	0.8^{ns}	0.3	11.9^{ns}	10.1	−1.3^{ns}	−1.0	−2.8	−1.1	−3.5	−4.8	0.4^{ns}	0.5	−1.8^{ns}	−2.1	−4.2	−5.3
IT	−10.9	−4.4	23.2	19.7	15.6	12.3	−15.9	−6.5	5.8	7.9	0.8^{ns}	1.0	−0.5^{ns}	−0.6	4.5	5.8
NT	−1.4^{ns}	−0.6	−18.0^{ns}	−15.3	−13.8^{ns}	−10.9	−1.5	−0.6	6.0	8.2	1.1^{ns}	1.4	−1.9	−2.2	2.9	3.7

P = pond (20–30°C); T1 = tank (15–20°C); T2 = tank (20–25°C); D = Taiwan; I = Israel; N = NOVIT4 (GIFT-derived strain by RIA1); T = Thailand; Crosses = males × females; ns = non-significant ($P > 0.05$); Est. = estimate.

crossing of genetically diverse populations (strain, breed) is expected to result in greater heterosis than crossing of closely related populations. The negative heterotic effects in our study together with those reported in tilapias by Bentsen *et al.* (1998) and Pongthana *et al.* (2010) suggest that future breeding programme in this population could be based on additive genetic variation rather than through crossbreeding.

Overall, performance of the fish was significantly lower in tanks than in the pond culture environments ($P < 0.001$). Apparently there were re-rankings of strains with regard to their additive genetic effects between tank and pond environments. Therefore, the strain by culture facility (pond or tank) interaction could be significant for both body weight and survival rate. Pongthana *et al.* (2010) also reported that red tilapia exhibited faster growth in freshwater ponds than in saline water tanks, although the performance difference in this study was due to salinity levels (0 vs. 30 ppt). Furthermore, in our present study the expression of heterosis for body weight was also dissimilar in tank and pond cultures, although the magnitude of the difference between the two environments was only 9%. From a study in common carp, Wohlfarth (1993) proposed that the expression of non-additive genetic effects may be increased under environmental conditions causing low growth rates. Bentsen *et al.* (1998) tested the GIFT strain under a wide range of environments from backyard farming to intensive culture systems, but there was inconclusive indication of greater heterosis in environments in which the fish had lower growth than in other ones. In rohu carp (*Labeo rohita*), Gjerde *et al.* (2002) reported that the average level of heterosis for body weight was −6.4% and −31.8% in mono- and poly-culture respectively. It is likely that the expression of non-additive genetic components depend on individual species and environments to which the fish are subject.

Although the strong effect of pond vs. tank culture was observed, the high and close to one correlation of the additive genetic performance between tank environments indicate that strain by water temperature was likely not biologically important. This is likely due to the small discrepancy in water temperature (15–20 vs. 20–25°C) between the two tank treatments used in the present study. In a pedigreed population of Nile tilapia also reared in different water temperature environments (21, 23.5 and 30°C) in North Vietnam, Luan, Olesen and Kolstad (2010) reported a high genetic correlation for body weight between the environments (rg > 0.70), although the estimates should be treated with caution because the maternal and common environmental effects were not included in statistical models of analysis. Based on our results together with those reported by Luan *et al.* (2010), development of a separate breeding programme for the temperate environment of Northern Vietnam therefore is not justified. As the ambient temperature rarely drops below 10°C in winter in Northern Vietnam, improving cold tolerance of Nile tilapia is not the sole objective of our breeding programme. However, a large body of the literature reports that this trait (cold tolerance) is largely controlled by additive genetic effects in several tilapia populations such as in Egyptian and Ivory Coast strains of *O. niloticus* (Tave, Jayaprakas & Smitherman 1990), in *O. aureus*, *O. mossambicus* and *O. niloticus* (Behrends *et al.* 1990), in GIFT, Sudan and Egypt (Sifa *et al.* 2002) or in seven divergent lines of tilapia (Armas-Rosales 2006). The heterotic effects were also not significant in these studies, but the maternal component could be important for body traits.

In conclusion, there are large additive genetic effects among strains involved in the diallel cross of the present study, suggesting that performance of the present Nile population can be improved by a wise choice of strain or by selective breeding. The level of heterosis was low and not different from zero for both body weight and survival rate. There were generally no re-rankings of strains between the two tanks (15–20 vs. 20–25°C). However, considerable differences in both performance and survival between pond and tank environments suggest that this effect should be accounted for in future breeding programmes.

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