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Estimation of genetic parameters for growth related traits at different stages of development in *Paralichthys olivaceus* (Temminck & Schlegel, 1846)

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ABSTRACT

Genetic parameters of growth traits at different growing stages of *Paralichthys olivaceus* (Temminck and Schlegel, 1846) were estimated using 30 families. The estimates of heritability for body weight at four developmental stages *viz*., 210, 450, 720 and 830 days of age, varied from 0.12 to 0.37. The heritability at 720 days of age was the lowest and that of 450 days was the highest. The maternal effect was 0.08 at 210 days, 0.03 at 450 days and almost zero (8.89 E-8 and 2.40 E-7) at 720 and 830 days. Correlation coefficients of the estimated breeding value (EBV) and phenotypic value (PV) of body weight at different developmental stages were 0.470 - 0.803, which were highly significant ($p<0.01$). Correlation of estimated breeding value (EBV) and phenotypic value (PV) was the highest at 210 days of age and the lowest at 720 days. Genetic correlation among the four stages showed large variation (0.339-0.811), which were highly significant except that of 210 and 830 days. The value and accuracy of estimating genetic parameters for growth traits at different stages was different. Therefore, the growth stage should be taken into account when designing a breeding program for growth traits in *P. olivaceus*.

Keywords: Breeding value, Growth related trait, Heritability, *Paralichthys olivaceus*

Introduction

Paralichthys olivaceus is one of the most important species for mariculture (Liu *et al*., 2005b), which shows morphological left-right asymmetry (Fan *et al*., 2015). *P. olivaceus* is widely cultured throughout the coastal areas of north China and the farming of the species is a rapidly growing industry (Song *et al*., 2012; Yang *et al*., 2013). As a result of long-term, high-intensity stocking and resource management (Shao *et al*., 2015), the farming of *P. olivaceus* in China is beset with many serious issues such as poor quality of seed, slow growth and increased susceptibility to diseases. This situation has affected profitability of fish farmers and constrained the development of *P. olivaceus* aquaculture in China (Liu *et al*., 2005a). In order to render the aquaculture development of *P. olivaceus* healthy and sustainable, it is necessary to breed *P. olivaceus* strains for faster growth.

In aquaculture production, the rate of growth is a critically important economic trait (Liu *et al*., 2011). The main objective of most selective breeding programs in fish is to enhance the growth rate, which can lead to more efficient fish production (Charo-Karisa *et al*., 2007). Breeding programs for growth traits in aquaculture typically use the family rearing of fry until the tagging size is reached. Thereafter, the tagged fish are mixed and reared in ponds until broodstock are selected (Blonk *et al*., 2010). Estimation of genetic parameters is the basis for selective breeding and planning of breeding programmes (Zhang *et al*., 2011). Genetic parameter estimates for growth-related traits have been reported in certain fish, *viz., Dicentrarchus labrax* (Karahan *et al*., 2013), *Salmo salar* (Guy *et al*., 2009), *Larimichthys crocea* (Liu *et al*., 2013), *Oncorhynchus mykiss* (Sae-Lim *et al*., 2013; Hu *et al*., 2013), *Hypophthalmichthys molitrix* (Gheyas *et al*., 2009), *Oreochromis niloticus* (Trọng *et al*., 2013; He *et al*., 2015), *Cyprinus carpio* (Ninh *et al*., 2011) and *Labeo rohita* (Das Mahapatra *et al*., 2007). Determination of the estimated breeding values (EBVs) of the breeding candidates and genetic parameters of target traits for captive bred fish at present are commonly based on the restricted maximum likelihood (REML) and best linear unbiased prediction (BLUP).

Liu *et al.* (2013) estimated the genetic parameters for the growth related traits of *P. olivaceus* (450 days old), based on the data collected from 24 families, but they did not study the genetic parameter at different ages. Therefore, the present study, focused on the growth traits of the *P. olivaceus*, at 210, 450, 730 and 830 days of age, which roughly corresponded to the conditions *viz.,* before overwintering, the first growth spurt, after overwintering and the second growth spurt, respectively. Heritability and EBVs of the body weight were estimated based on the data collected from 30 families produced by artificial fertilisation in 2010. We investigated changes in the genetic parameters and EBVs of the body weight of *P. olivaceus* at different ages and analysed the correlation between ages to unravel the relationship between body weight at different growing stages, which can provide important reference material to carry out selective breeding of *P. olivaceus*.

Materials and methods

Broodfishes for the study were derived from 5 lines: Korea stock (KS) and four selected lines (F0719, F0750, F0751 and F0768) produced in 2007, as described by Tian *et al*. (2011). All broodfishes were cultured in the Haiyang Aquaculture Breeding Station, Shandong Province, China. Artificial insemination was employed to breed *P. olivaceus* in late April 2010. One male was mated with one/three females (for production of full sibs/paternal half-sibs) and one female also was mated with one/three different males (full sibs/maternal half-sibs). The mating lasted about one month. Fertilised eggs were kept in separate tanks (3 m^3) with flow through water. The water temperature of the tanks ranged from 17 to 25°C with a salinity of 30 - 32‰. The pH of seawater was about 7.8. Thirty families were obtained by crossing 25 female and 16 male parents (Table 1) and data on these were analysed. Families were reared separately in tanks. Each tank was stocked with about 1500 fry. Transformed fry from floating to demersal state (about 20 days old) were selected for stocking.When the youngest fry were 80 days old, all families were tagged with Visible Implant Fluorescent Elastomer (VIE) tags. About 150 to 200 individuals were randomly sampled from each family and tagged. In total, 4753 individuals were tagged and stocked randomly in two indoor ponds (40 m^3).

Table 1. The breeding design using the five broodstocks along with the No. of family

Female	Male					
	F0719	F0750	F0751	F0768	KS	Total
F0719	1	1	2	1	2	7
F0750	1	1		2	1	5
F0751	1	1	1		7	10
F0768				1	1	2
KS		5			1	6
Total	3	8	3	4	12	30

Data collection

Genetic parameters were estimated for the following four growing stages *viz.,* i) before overwintering, when the fish were approximately 210 days and were just in the suitable temperature condition to grow; ii) the first growth spurt, when the fish were approximately 450 days old and were in the suitable temperature condition to grow; iii) after overwintering, when the fish were 720 days old and were in the low temperature condition which slows down growth; and iv) the second growth spurt, when the fish were approximately 830 days old and were in the suitable temperature condition to grow. About 28 - 50 individuals were randomly sampled from each family and the body weight (g) measured. The data thus recorded were used to estimate genetic parameters and predict the estimated breeding value (EBV).

Statistical analysis

Dataset was analysed using SAS software (SAS, 2011). The variance components of body weight in the four growth stages were estimated using REML and BLUP in the ASReml software (Gilmour *et al*., 2012). The mixed model applied to analyse the body weight consisted of the fixed effects (the rearing ponds) and random effect (the animal and maternal effect). The full-sib effect included in the model will lead the animal effect variance to be almost zero, and the paternal effect variance was also almost zero. Therefore, the full-sib effect and paternal effect had not been added in the model. Thus, the model was written as:

$$y = \mu + pond + age + a + m + e$$

where, y is the vector of body weight; μ is the overall mean; *pond* indicates the effect of pond (fixed); *age* is the age in nimber of days of the family fixed as covariate in the analysis model; a is the random effect of each animal, $a \sim (0, A\sigma_a^2)$, where A is the additive genetic relationship matrix among all individuals, including their parents without data records of body weight; m is the maternal effect, fixed as a random effect and e is the residual effect.

The heritability (h^2) and maternal effect (h_m^2) of the body weight within each age group were computed as:

$$h^2 = V_a / (V_a + V_m + V_e)$$

$$h_m^2 = V_m / (V_a + V_m + V_e)$$

where, V_a is the random effect variance; V_m is the maternal effect variance; and V_e is the residual variance.

Results and discussion

Descriptive statistics

The details of body weights at the four different ages along with the number of family, sampling number from each family and total number of records at each developmental age studied are provided in Table 2. As expected, the mean values increased, and the coefficient of variation declined as the fish grew, which means the individual difference in body weight decreased with age. The descriptive statistics of data from ponds are summarised in Table 3. In the beginning, fish were reared in two ponds, and thereafter divided and stocked into three ponds after the second measurement, to avoid high stocking density and to facilitate growth. Comparison of the mean values of body weight in separate ponds in each growth stage, indicated significant differences ($p<0.05$) between the ponds within each age group. This showed that the pond effect need to be included in the analysis model as a fixed effect.

Table 4. Heritability estimates for body weight and maternal effects (±SE)

Age in days	h^2	h_m^2
210	0.32 ± 0.18	0.08 ± 0.08
450	0.37 ± 0.19	0.03 ± 0.08
720	0.12 ± 0.05	8.89E-8
830	0.22 ± 0.07	2.40E-7

h^2: trait heritability; h_m^2: the maternal effect

There are numerous reports on the heritability estimates for growth related traits of cultured fish. The heritability estimates in the present study ranged from 0.12 to 0.37 which is comparable to those reported by Navarro *et al.* (2009). Whatmore *et al*. (2013) for body and carcass traits in *Seriola lalandi* (0.15 to 0.30). Gjerde *et al*. (2012) estimated the heritability of body weight in *Oreochromis niloticus* (0.19±0.04), which was higher than thepresent estimate at 720 days of age (0.12) but lower than those for the other three ages (0.22 - 0.37) obtained in the present study. In the study of Shimada *et al*. (2007), the estimates of heritability for body weight in progeny from wild caught and repeatedly crossbred *P. olivaceus* broodfish were 0.499 and 0.385, respectively. Tian *et al*. (2011) also reported estimates of heritability in *P. olivaceus* as 0.48 ± 0.04 for body weight at 8 months of age. In this study, the estimates of heritability for body weight at the four different ages (0.12 - 0.37) were somewhat lower compared to the above two reports. The observed differences could be attributed to the different statistical model used and method of parameter estimation as well as the difference

Table 2. Summary of measurement for *P. olivaceus* at four different ages

Age in days	No. of family	Sample no. from families		No. of records	Min. (g)	Max. (g)	Mean (± SE)	C.V. (%)
		Minimum	Maximum					
210	30	45	50	1450	12.8	220.0	83.46 ± 0.60	45.72
450	30	40	50	1305	12.2	362.2	171.07 ± 1.12	34.16
720	30	35	50	1336	101.6	923.4	413.74 ± 3.55	31.73
830	30	28	48	1292	120.4	1307.2	599.64 ± 4.62	28.05

Table 3. The descriptive statistics for body weight in each pond at four different ages

Age in days	Pond	Number	Body weight (g)			
			Min.	Max.	Mean (±SE)*	C.V. (%)
210	1	655	12.9	220.0	89.88 ± 0.91[a]	43.35
	2	795	12.8	215.7	78.17 ± 0.78[b]	46.90
450	1	581	12.2	362.1	207.88 ± 1.31[a]	21.90
	2	724	19.5	362.2	141.54 ± 1.29[b]	35.55
730	1	485	172.4	923.4	496.08 ± 5.51[a]	24.63
	2	382	238.8	736.0	458.26 ± 3.87[b]	16.80
	3	469	101.6	546.0	292.34 ± 3.35[c]	25.36
830	1	450	226.2	1307.2	654.73 ± 7.03[a]	22.79
	2	380	267.2	1251.4	701.67 ± 7.03[b]	19.95
	3	462	120.4	777.2	462.06 ± 4.76[c]	22.64

*Comparison of the mean values of body weight between ponds in each stage. Means bearing different superscripts are significantly different ($p<0.05$)

Heritability estimation

Determination of the heritability for economically important traits provides the foundation for designing suitable breeding programme. Variance components, heritability of body weight (h^2) and heritability of maternal effect (h_m^2) were estimated (Table 4). The heritability for body weight ranged from 0.12 to 0.37, showing a large variation. The heritability of body weight at 720 days of age (0.12) was less than those of the other three ages. One of the probable reasons is that 720 days of age *P. olivaceus* were in a stage of overwintering with low - temperature environment, and were in a state of growth arrest. Additionally, the estimated heritability at the first growth spurt (450 days of age) was larger than that of the second growth spurt (830 days of age), which indicated that heritability estimates in the early growing stage of fish were higher than that in the later stages under the same conditions.

in the sample size and data structure, as reported by Zhang *et al*. (2014).

Maternal effect was present during the first two ages (0.08 and 0.03 respectively), whereas these effects were not discernible at the latter two growing stages, which were different from the value of maternal effect (0.07 - 0.54) obtained by Liu *et al*. (2011). This is as expected because as the fish grows, the maternal influence diminishes. Therefore, maternal effect would be important and should be considered while evaluating growth traits during early life stages of *P. olivaceus*.

Correlation analysis

ASReml software can estimate variance components in addition to solving mixed linear model equations to predict EBV for body weight of all individuals. The fish used as broodstock in a breeding program should be selected from the highest ranked breeding value of candidates (Luan *et al*., 2012). Therefore, it is of crucial importance that the individual breeding value should be predicted as accurately as possible. There are two principal factors which impact the accuracy of EBVs: the amount of the available information on relatives of the candidate and the statistical method used to predict the EBV. The correlation between EBV and phenotypic value (PV) is regarded as an important index in measuring EBV prediction accuracy. Hence, these correlations were calculated and are presented in Table 5. The correlation between PV and EBV at all the four ages ranged from 0.470 to 0.803 and there was no definite trend visible.

The correlation between PV and EBV of body weight at 210 days of age (0.803) was the highest among the four ages and that of 720 days of age (0.470) was the lowest, which indicated that the third age (during overwintering) was not an appropriate stage to carry out genetic evaluation and selection of broodstocks. Additionally, all the correlations between EBV and PV of body weight were highly significant at all the four ages ($p<0.01$). The correlation between EBV and PV fluctuated between the four ages (0.470 - 0.803), indicating that the prediction accuracy of breeding value was different between different stages. These estimates are lower than the estimates by Zhang *et al*. (2011), who reported genetic correlations of 0.732 - 0.971 between EBV and PV of body mass in shrimp, *Fenneropenaeus chinensis*. Among the four ages, the correlation between PV and EBV at 720 days of age (0.470) was obviously less than those of the other three ages, due to the comparatively low heritability of body weight, from which it can be inferred that selection of broodstock carried out in the growth arrest stage is unsuitable.

Table 5 depicts the correlation of the family breeding values among the four ages for body weight (off-diagonal). A large difference of 0.339-0.811 was observed among the estimates of correlation. They were highly significant ($p<0.01$), except the correlation between 210 and 830 days of age. The correlation coefficient of the first age and its adjacent age (0.811) is higher than those of the rest (0.569 and 0.339) of the ages, which suggested the growth at one stage is correlated to its previous one.

Table 5. The family breeding value - correlation among the four stages of the body weight and the correlations of estimated breeding value and phenotypic value in the four stages [a]

Age in days	210	450	720	830
210	0.803**			
450	0.811**	0.705**		
720	0.569**	0.636**	0.470**	
830	0.339	0.564**	0.612**	0.569**

[a]The diagonal in the table are the correlation coefficients of the estimated breeding value and phenotypic value and the lower triangles for the body weight; ** Correlation is significant at 0.01 level (2 - tailed).

This study used only the information from parents and their F1 generation, which are not optimal data as far as the data structure for genetic analysis is concerned. Therefore, additional information (*i.e.* data from other generations, collateral relatives) should be collected and used in order to increase the accuracy and precision of the estimation for the genetic parameters.

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