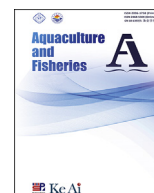




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Original research article

Heritability of growth traits in the Asian seabass (*Lates calcarifer*)

Baoqing Ye^a, Ziyi Wan^a, Le Wang^a, Hongyan Pang^a, Yanfei Wen^a, Huiming Liu^a,
Bing Liang^b, Huan Sein Lim^b, Junhui Jiang^b, Genhua Yue^{a, c, d, *}

^a Molecular Population Genetics and Breeding Group, Temasek Life Sciences Laboratory, Singapore 117604, Singapore

^b Agri-Food and Veterinary Authority of Singapore, 5 Maxwell Road, Singapore 069110, Singapore

^c Department of Biological Sciences, National University of Singapore, Singapore 117543, Singapore

^d School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore

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ABSTRACT

Growth is an economically important trait in aquaculture. To improve growth trait of the Asian seabass (*Lates calcarifer*) we have been carrying out, since 2004, a selective breeding program. This study focuses on growth traits in the F₂ fish generation, comprised of offspring from 23 mass crosses from 383 F₁ brooders. Using genotyping analysis for 10 microsatellites from both brood stock and progeny we have reconstructed the pedigree of each mass-cross. For F₂ generation at 90 days post hatch (dph), we have recorded body weight (BW) for 12,117 individuals and total length (TL), standard length (SL) and condition factor (K_H and K_{SL}) for 3530 individuals; and all five traits for 2136 individuals at 270 dph. At 90 dph the average BW was 46.88 ± 20.95 g. Combining pedigree information, recorded growth traits and Restricted Maximum Likelihood method, we have estimated that the narrow sense heritability (h^2) in F₁ fish for BW, TL, SL, K_H and K_{SL} was at, 90 dph, 0.12 ± 0.03 , 0.11 ± 0.03 , 0.10 ± 0.03 , 0.20 ± 0.04 and 0.11 ± 0.03 , respectively and, at 270 dph, 0.34 ± 0.07 , 0.32 ± 0.07 , 0.30 ± 0.06 , 0.13 ± 0.04 and 0.11 ± 0.04 , respectively. At 90 dph the realised heritability for BW was 0.13. Comparing with F₁ generation, the growth performance of F₂ fish was increased by 14.4%. Heritability of growth traits will be useful for future genetic improvement programmes of the Asian seabass.

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1. Introduction

Growth is one of the most important traits in fish aquaculture (Gjedrem, 2005). Heritability is the measure of the relative proportion of genetic versus environmental factors that determine the total variation of a specific trait. Heritability is an important genetic parameter in selective breeding programs (Gjedrem & Baranski, 2010). Specifically, narrow sense heritability, which is the proportion of total phenotypic variation due to additive genetic factors, is important in predicting how a trait will respond to selection. Heritabilities have been estimated for a number of traits in several aquaculture fish species, such as growth in the common carp (*Cyprinus carpio*, Vandeputte et al., 2004), and Asian seabass (*Lates calcarifer*, Wang et al., 2008, Domingos et al., 2013); resistance to columnaris disease in the Atlantic salmon (*Salmo salar*, Evenhuis, Leeds, Marancik, LaPatra, & Wiens, 2015) and others (Gjedrem,

Robinson, & Rye, 2012). In aquaculture species the estimated heritabilities for growth traits range from 0 to 0.83 (Gjedrem, 1983; Gjedrem & Baranski, 2010). Heritability estimates are affected by a number of factors, such as, genetic background, genetic variation, sample size, culturing conditions and the number of generations of selection that the population has gone through (Gjedrem, 2005; Visscher, Hill, & Wray, 2008). In aquaculture species the genetic gain for growth rate is greater than 12% per generation (Gjedrem & Robinson, 2014). However, for newly emerging species, information about heritabilities for important traits and increase of growth is still limited.

The Asian seabass (*Lates calcarifer*) is an important commercial fish in the Indo-Pacific region. It is considered an ideal aquaculture species as it is in high demand, grows rapidly and can grow in salinities ranging from fresh to sea water and thus can be raised in monsoonal areas (Jerry, 2013; Yue, Li, & Orban, 2001). According to the United Nations Food and Agricultural Organisation's, there is a recognized need for genetic selection programmes for Asian seabass to target faster growth and disease resistance. However to date little efforts have been made (Rimmer, 2006). A unique study

* Corresponding author. Molecular Population Genetics and Breeding Group, Temasek Life Sciences Laboratory, Singapore 117604, Singapore.
E-mail address: genhua@tlsl.org.sg (G. Yue).

describing significant heritabilities and high phenotypic variation in body weight and length of Asian seabass at very early stages (21 dph to 80 dph) suggested that it would be possible to improve fish growth rate through selection at an early stage (Chandra, Kailasam, Thirunavukkarasu, & Abraham, 2000). Since 2004, our laboratory, in collaboration with the Marine Aquaculture Center, Agri-Food and Veterinary Authority of Singapore, has been carrying out an Asian seabass selective breeding programme to improve growth and disease resistance using an initial broodstock composed of more than 500 adults from Malaysia, Indonesia, Thailand and Singapore (Wang et al., 2008; Yue et al., 2009). Using the growth traits from the first two batches (1704 individuals) of the first generation progeny (i.e. F_1) produced from the broodstock, a genetic study was carried out focused on estimating the narrow-sense heritabilities of Asian seabass growth traits (body weight, standard length and Fulton's condition factor based on standard length) at 90 dph (days post hatch), and the correlation of each trait between 90 dph and 270 dph (Wang et al., 2008). This study concluded that the heritabilities for growth traits at 90 dph ranged from 0.15 to 0.31 and that maternal and dominance effects were negligible and that the correlation of each trait between 90 dph and 270 dph was strong. These findings supported the hypothesis that fish selection could be performed at an early age (i.e. 90 dph), hence saving maintenance costs (Wang et al., 2008). Further support to the previous findings came from a recent study on the Australian strains of Asian seabass, in which a different set of growth traits, wider range of ages and habitats and estimating the genotype by environment ($G \times E$) effects were considered and found that $G \times E$ effects were mostly negligible (Domingos et al., 2013). Both studies used the first generation (F_1) of Asian seabass raised in captivity to estimate the heritabilities of the initial broodstock (F_0). It is well known that in breeding programs, heritability estimates vary (are reduced) from generation to generation due to the reduction of genetic variation (Gjedrem, 2005). It is essential to estimate heritability for a selected species in each generation to ensure enough selective response. The purpose of this study was to estimate narrow-sense heritabilities (including realised heritability) for growth traits at 90 dph and 270 dph from F_1 to F_2 generation of Asian seabass to facilitate future genetic improvement. To our knowledge, this is the first study that considers the second generation (i.e. F_2) of selectively bred Asian seabass to estimate the heritabilities of growth traits in F_1 , and comparing these heritabilities to F_0 .

2. Materials and methods

2.1. F_1 brooders, spawning, culturing F_2 and measuring growth traits

A total of 383 sexually mature individuals selected from over 15,000 F_1 individuals were used as brooders to produce F_2 for further selection. These brooders were grouped into three groups with approximately an equal number of individuals that were genetically selected based on 10 microsatellite markers (*Lca08*, *Lca20*, *Lca21*, *Lca58*, *Lca64*, *Lca69*, *Lca70*, *Lca74*, *Lca98* and *PAR*) (Zhu et al., 2010). To avoid inbreeding, within each group, males and females were genetically distantly related. To produce each batch (i.e. offspring of a mass cross) of F_2 fish, 10 to 15 sexually matured males and females (i.e. up to 30 individuals) from the same brooder group were selected as parents. The number of parents and chosen groups depended on the physical conditions for spawning and sex composition of every group. As Asian seabass is a protandrous hermaphrodite, with a range of ages at which sex inversion takes place (De Jesus-Ayson & Ayson, 2014), the male: female ratio from each brooder group changed throughout the study. Also, each brooder could be involved

in more than one mass cross. The selected individuals for crosses were induced hormonally during their natural spawning rhythm and placed in a 60-tonne tank for mass spawning. A total of 23 mass crosses were carried out that resulted in 23 batches of offspring. Growth traits, including body weight (*BW*), total length (*TL*), standard length (*SL*), Fulton's coefficient of condition based on *TL* (i.e. K_{tl}) and *SL* (i.e. K_{sl}), were measured in fish at the 90 and 270 dph, respectively. These stages were chosen due to their importance in the aquaculture of Asian seabass as 90 dph is the age from which physical measurements can be practically taken and 270 dph is the harvesting age in Southeast Asia.

Eggs from every mass cross were collected on the second night of spawning and transferred to 1-tonne incubation tanks to hatch. Approximately 60,000 eggs were randomly collected and hatched from each cross. The remaining eggs were used for other studies. The 60,000 larvae were cultured to 26 days post hatch (dph) according to standard protocols (Wang et al., 2008), with slight modifications in terms of grading: in this study, juveniles were size graded every week from 26 to 60 dph (instead of 30–65 dph) and a total of 5 gradings were performed. Each time, the smallest one-third of the fish were culled and for each batch, the larger juveniles (3000 individuals per batch) were kept and raised in cylindrical 7-tonne capacity tanks. Grading is necessary due to the cannibalistic nature of the Asian seabass (Ayson, Sugama, Yashiro, & de Jesus-Ayson, 2014). As each brooder could be involved in more than one mass cross, its offspring could be assigned to more than one tank. Since each mass cross involved 20 to 30 brooders there would be offspring from several distinct families per tank.

At 90 dph, for each mass cross, 500–600 fish were randomly collected and growth traits measured to assess growth performance. Then, among all the 3000 individuals in the batch, the largest individuals (up to 380 per batch) were tagged with RFID tags, and body weight (*BW*), total length (*TL*) and standard length (*SL*, i.e. total length excluding the length of the caudal fin) were measured. Fulton's coefficient of condition based on *TL* (i.e. K_{tl}) and *SL* (i.e. K_{sl}) were calculated according to the following formula (Nash, Valencia, & Geffen, 2006):

$$K_{tl} = 100(BW)/(TL/10)^3$$

$$K_{sl} = 100(BW)/(SL/10)^3$$

where *BW* is in grams (g), and *TL* and *SL* are in millimetres (mm). Then, the second dorsal fin of each tagged fish was clipped and stored in absolute ethanol for DNA extraction and genotyping analysis for 10 microsatellites (see details below). The non-tagged fish were not genotyped. The tagged fish were raised to adulthood. Every 90 days, their measurements were taken and Fulton's coefficients of condition was calculated. Up to 270 dph, each batch of fish was raised in the same tank.

2.2. Inter-trait and cross-age correlations

The phenotypic and genetic correlations between the various traits (i.e. inter-trait correlations) were calculated to estimate the effect of selection for one trait in relation to other traits. In addition, the phenotypic and genetic correlations for each trait between 90 dph and 270 dph (i.e. cross-age correlations) were calculated to gauge the potential accuracy of predicting the growth performance of fish at marketing age according to growth performance at 90 dph. In each case, the correlation, *r*, was calculated as,

$$r = \text{cov}(a,b)/\sqrt{\text{var}(a), \text{var}(b)}.$$

In the case of phenotypic correlation, a and b would be the corresponding phenotypic values for the two traits, whereas for genetic correlation, a and b would be the corresponding breeding values for the two traits, calculated using ASReml 3.0 (see section 2.5 Estimation of heritabilities for growth traits by REML). For cross-age correlations, the corresponding values at 90 and 270 dph for one trait were treated as values for two different traits. Statistical significance was calculated using a t -test and p -value significance thresholds considered are: $\alpha = 0.01$ and $\alpha = 0.05$. The records used in the calculation of phenotypic correlations include all individuals with traits data, whereas the records used in the calculation of genetic correlations include all individuals with known parents.

2.3. Pedigree reconstruction using microsatellite genotyping

To reconstruct the pedigree of F_1 and F_2 , all selected individuals (including the F_1 fish chosen to produce F_2) were genotyped for 10 microsatellites (*Lca08*, *Lca20*, *Lca21*, *Lca58*, *Lca64*, *Lca69*, *Lca70*, *Lca74*, *Lca98* and *PAR*) (Zhu et al., 2010). Genomic DNA from parents and offspring was isolated according to Yue and Orban (2005). For microsatellite amplifications, PCR was conducted on a BioRad T-100 machine using the following programme: 94 °C for 2 min, followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s. This was followed by a final extension of 72 °C for 5 min. Final PCR reaction volume was 25 μ l, and consisted of 1xPCR buffer (Finnzymes, Espoo, Finland), 1.5 mM of $MgCl_2$, 200 nM of each PCR primers, 50 μ M of each dNTP, 40 ng genomic DNA and 1 U of DNA Taq-polymerase (Finnzymes, Espoo, Finland). The PCR products were checked on 1% agarose gels stained with ethidium bromide (0.0001% by volume) and genotyped on an ABI3730xl DNA sequencer (Applied Biosystem, Foster City, CA, USA). The PCR product fragment sizes were quantified using the ROX-500 size standards (Applied Biosystem, Foster City, CA, USA) with the software GENEMAPPER version 3.5 (Applied Biosystem, Foster City, CA, USA). The genotypes of the offspring were converted to suitable input format and subsequently analysed with the software PAPA v2.0 (Duchesne, Doddout, & Bernatchez, 2002) for parentage assignment with zero error model based on sexual reproduction using the genotype of all parents.

2.4. Realised heritabilities

Since the F_1 and F_2 fish were cultured under similar conditions in tanks in Marine Aquaculture Center, Singapore, we also estimated the realised heritability for growth traits from F_1 to F_2 generation. Realised heritability for a trait across F_1 and F_2 was calculated based on the following equation (Tave, 1986):

$$\text{Realised } h^2 = R/S$$

where R = response to selection, i.e. (overall mean for F_2 fish) – (overall mean for F_1 fish) and S = selection differential, i.e. (mean for F_1 fish selected to produce F_2) – (overall mean for F_1 fish).

This estimation does not depend on pedigree and thus also include records for individuals which had not been assigned sire (male parent), dam (female parent) or both. On the other hand, it required random sampling from the overall populations of both F_1 and F_2 fish at 90 dph, in order to estimate the phenotypic means for a population. Based on the availability of data, realised h^2 was calculated for BW at 90 dph. There were insufficient trait records at 270 dph in F_1 for the realised heritability at 270 dph to be estimated.

2.5. Estimation of heritabilities for growth traits by REML

In the previous study by Wang et al. (2008), the heritability depending on pedigree was estimated using an Analysis of Variance (ANOVA) method. However, in this study, we have chosen to use a Restricted Maximum Likelihood (REML) method. REML is an iterative method that firstly assumes the variance components to be certain values (e.g. by a rough guess). Thereafter, each iteration has two basic steps: 1. The current assumed variance components are used to solve the model equation; 2. Based on how the solution fits the actual data (indicated by a special type of likelihood function), the variance component estimates are updated, to be used in step 1 of the next iteration. These two steps are repeated until the estimates converge (Gjedrem, 2005). This method has known advantages over ANOVA, some of which are especially relevant to this study. Firstly, REML can account for selection. Simulation studies have shown that it can minimize bias due to phenotypic selection provided that the pedigree does not have much missing information (Schenkel & Schaeffer, 2000) and dataset is not too small (Duangjinda, Misztal, Bertrand, & Tsuruta, 2001). Secondly, REML can make use of any relationship between the individuals. These make REML especially useful to this study, as most of the data was collected after selection, and the use of the mass-cross method of spawning would result in a random pedigree structure which is more difficult for ANOVA to handle.

REML has already been used to estimate genetic parameters, including heritability, of growth traits for Asian seabass (Domingos et al., 2013), as well as a few other economically important fish such as Nile tilapia, *Oreochromis niloticus* (Khaw, Ponzoni, Yee, & bin AzizBijma, 2016), gilthead seabream, *Sparus aurata* (Navarro et al., 2009) and Atlantic salmon, *Salmo salar* (Quinton, McMillan, & Glebe, 2005).

For the estimation of pedigree-based heritability, only records of fish belonging to any batch that shared at least one parent with another batch were chosen. This was to ensure that the environmental and genetic factors were crossed rather than nested, and thus the variances could be easily separated from each other (Schielzeth & Nakagawa, 2013). Records of fish with unidentified parents were excluded. The chosen records were then converted into a suitable input format and input into the software ASReml 3.0 (VSN International Ltd, UK) to perform univariate analysis on each trait (i.e. BW , TL , SL , K_{fl} or K_{sl}) using the Restricted Maximum Likelihood (REML) method.

For each trait, the effects on the phenotype were fitted according to an Animal model (Wilson et al., 2010), as represented in the following Mixed Model Equation:

$$y = Xb + Za + e \quad (1)$$

y is the vector of phenotypic observations, b is the vector of common environment effects. In this case, each batch corresponds to one common environment (i.e. one level in the factor), since each batch of fish was raised together in one separate tank up to 270 dph. a is the vector of additive genetic (animal) effects. e is the vector of residual effects. b are fixed effects, while a and e are random. X and Z are the incidence matrices relating observations to the batch and animal effects, respectively. Since the common environment is mostly under the control of the breeder, it is considered a fixed effect, and under the REML procedure, it does not contribute to the phenotypic variance, V_p . Hence, the latter would only be partitioned into the following variance components:

$$V_p = V_A + V_E \quad (2)$$

where V_A is the variance due to animal effects and V_E is variance

due to the residual effects, and the heritability for each trait would be estimated based on:

$$h^2 = V_A/V_P$$

Hence, the standard error (SE) of h^2 was the standard error of V_A/V_P .

To confirm the estimation results from ASReml, the same genetic analyses were carried out using the software Wombat (Meyer, 2007). Other effects, such as paternal and maternal environment effects had also been considered. However, it was found that these additional effects tended to increase the corrected Akaike Information Criterion (AICc) and the Bayesian Information Criterion (BIC) associated with the model when added to it, indicating that they deplore the fit of the model to the data (Burnham & Anderson, 2004). AICc is similar to the usual Akaike Information Criterion (AIC), except for being corrected to be more suitable for finite samples. On the other hand, removing the batch effect from the model had a similar consequence. Hence the current model as represented in Equation (1) was chosen as the optimal model. For each trait, besides the variance components, ASReml 3.0 also estimates the breeding value for each individual in the form of Best Linear Unbiased Prediction (BLUP) solutions. These would be used to estimate the genetic correlations (see section 2.2 *Inter-trait and cross-age correlations*).

3. Results and discussion

3.1. Growth performance of F₂ Asian seabass

The mean BW at 90 dph of the F₂ fish, measured from 12,117 individuals from 23 mass crosses, was 46.88 ± 20.95 g; whereas the average BW at 90 dph of the F₁ fish, obtained from 17,155 individuals derived from 39 mass crosses of 453 founders, was 40.98 ± 23.09 g. The F₁ and F₂ fish were cultured under similar density, feeding scheme, temperature, water salinity, tank model, lighting and water circulation. Hence it can be inferred that at 90 dph there was a 14.4% increase in the average BW, a noticeable improvement in growth performance, from F₁ to F₂. Similar improvement of growth performances in selective breeding programs were previously reported for the Kuruma prawn, (*Penaeus japonicus*, 13%) (Hetzel, Crocos, Davis, Moore, & Preston, 2000), coho salmon (*Oncorhynchus kisutch*, 15%) (Hershberger, Myers, Iwamoto, McAuley, & Saxton, 1990) and many other aquaculture species (Janssen, Chavanne, Berentsen, & Komen, 2016). In this study, at 270 dph, the mean BW of F₂ fish, measured from 2136 individuals, was 505.99 ± 190.88 g (Table 1). For a fairer comparison with F₁, for which we only had 270 dph BW data for the largest 187 selected individuals, we have also estimated the average 270 dph BW of the largest 187 selected F₂ individuals and found to be 877.89 ± 118.41 g higher than the estimated (690.25 ± 185.14 g) from the 187 F₁ individuals. The data suggests that there is an increase of 27.2% in fish BW at 270 dph. However, this comparison should be taken with caution, since it is based on a small number of fish, and the result may reflect larger phenotypic variance of the present generation. Furthermore, besides genetic factors, it is possible that BW increase in F₂ fish when compared with F₁ fish could also be due to the improvement of management. A fairer comparison of growth performances between F₁ and F₂ fish should be conducted under the same environmental condition and same time period. However, in practice, it is difficult to conduct due to the long generation interval (>4 years), if two generations of adult brooders are kept, the older generation will be too old to produce high quality eggs.

3.2. Parental assignment using microsatellite genotyping

From 23 batches of mass crosses, out of 4036 F₂ fish genotyped, 3871 (95.8%) were successfully assigned both sire and dam (Table 1). Every batch shared common parents with other batches. The rate of successful parentage assignment is similar to the data for F₁ Asian seabass fish previously reported (Wang et al., 2008) (Domingos et al., 2013; Gheyas et al., 2009; Vandeputte, 2003). Therefore, we believe that an overall rate of over 95% successful assignment is good enough for a practical breeding program. Certainly, further improvement of the successful rate of parentage assignment is possible, but requires additional costs and time (Yue & Xia, 2014). Furthermore Table 1 shows that the number of families remaining in each batch at 90 dph ranged from 3 to 27, with an average of 17.5, and a total of 279 distinct families for the whole data set. Hence, we believe that in our study the brooder contribution and genetic variability is sufficient as there is more than one family in each batch.

3.3. Inter-trait and cross-age correlations

The phenotypic (r_p) and genetic (r_g) correlations are shown in Table 2 and Table 3, respectively. In general, for inter-trait correlations, both phenotypic and genetic correlations seem to follow a similar pattern. At the same age, the inter-trait correlations of BW, TL and SL are strong, positive ($r_p, r_g > 0.8$), and statistically significant ($p > 0.01$), for all the data sets. On the other hand, the correlation between BW, TL or SL and Fulton's condition factor (K_{tl} or K_{sl}) range from weak negative ($r_p = -0.268, r_g = -0.215$) to weak positive ($r_p = 0.202, r_g = 0.231$). This suggests that within the three basic traits, improving one trait by selection is likely to produce a similar selection response in another trait, but doing so is unlikely to produce any systematic selection response in K_{tl} or K_{sl} . These data are similar to what was previously reported for the Asian seabass (Domingos et al., 2013; Wang et al., 2008) and other fish species, such as the gilthead seabream (*Sparus auratus* L.) (Garcia-Celdran et al., 2015; Navarro et al., 2009).

Interestingly, while phenotypic correlation between K_{tl} and K_{sl} at 90 dph is also very strong and positive ($r = 0.761$), it is less than BW, TL and SL. The phenotypic correlation between K_{tl} and K_{sl} at 270 dph is even smaller ($r = 0.490$). Both are also noticeably less than the genetic correlations between K_{tl} and K_{sl} ($r_g = 0.934$ at 90 dph; $r_g = 0.886$ at 270 dph). Hence, gauging one Fulton's condition factor using the other, would be more difficult than gauging one of the three basic traits (BW, TL or SL) using another. A possible reason for this is that Fulton's condition factor is inversely proportional to the cube of the total or standard length. Hence, any difference between the total or standard length would have been compounded when calculating the Fulton's condition factor based on the two lengths, resulting in a greater divergence between K_{tl} and K_{sl} than would be apparent from comparing TL and SL. The relatively high genetic correlations between K_{tl} and K_{sl} shows that the divergence in phenotypic values between the two traits is mostly due to environmental rather than genetic factors.

Unexpectedly, the cross-age correlations for BW, TL or SL found in this study ($r_p = 0.398, 0.413$ or 0.443 , respectively; $r_g = 0.534, 0.430$ and 0.398 , respectively), while still positive and statistically significant, are lower than the cross-age correlations for BW or TL found by Wang et al. ($r_p = 0.601$ or 0.553 , respectively). This seems to imply that, the extent to which selection at a younger age (e.g. 90 dph) would be effective in producing a similar selection response at harvest age (e.g. 270 dph), is slightly lower in F₂ than in F₁ generations. However, since the earlier study did not estimate cross-age genetic correlations, whether this decrease is genetic in nature or is purely due to environmental differences between the generations,

Table 1
Batches of Asian seabass used to estimate growth traits heritabilities.

F ₂ fish	90 dph				270 dph				Individuals assigned parents	PA%
Batch	Sires	Dams	Families	Records	Sires	Dams	Families	Records		
1	13	5	27	345	11	3	16	42	330	95.7
2	—				7	4	10	506	500	98.8
3	7	7	16	380	7	7	16	310	375	98.7
4	3	1	3	20	2	1	2	10	20	100
5	7	1	7	162	3	1	3	55	156	96.3
6	11	3	15	172	10	2	11	48	170	98.8
7	7	5	16	156	5	4	9	54	152	97.4
8	9	7	23	156	8	5	14	56	143	91.7
9	11	6	24	156	10	6	16	56	148	94.9
10	10	7	16	156	7	5	9	55	153	98.1
11	10	10	29	156	7	6	12	54	155	99.4
12	16	15	38	156	13	11	20	57	151	96.8
13	8	5	8	155	7	4	5	53	142	91.6
14	8	4	11	190	4	4	6	81	178	93.7
15	8	5	17	190	7	5	10	67	174	91.6
16	3	5	8	30	3	5	7	20	29	96.7
17	8	8	21	190	8	7	14	74	186	97.9
18	9	8	26	190	8	8	19	91	188	98.9
19	9	10	32	190	9	8	22	92	175	92.1
20	8	8	19	95	8	8	17	91	90	94.7
21	6	3	13	95	6	3	13	81	92	96.8
22	6	4	8	95	6	4	8	94	79	83.2
23	6	4	8	95	6	4	8	89	85	89.5
Min	3	1	3	20	2	1	2	10	20	83.2
Max	16	15	38	380	13	11	22	506	500	100
Mean	8.3	6.0	17.5	160.5	7.0	5	11.69	92.9	168.3	95.4
Overall	57	65	279	3530	54	59	202	2136	3871	95.8

Notes: The number of parents, families and records (each record is the data for one individual) involved in the REML-based estimation of heritability, as well as their parental assignment success rate (PA%) in the Asian seabass. Batch 2 was not used at 90 dph as the growth trait values were not recorded. The overall number of sires, dams or families is not the sum of all batches, as there were common parents and families across the batches. Also, since the F₂ fish used in this analysis was only a subset of that used for the estimation of realised heritability, the number of contributing parents was also much less than that (383) used for the estimation of realised heritability.

Table 2
Phenotypic correlation of growth traits in the Asian seabass.

Inter-trait correlations:		BW	TL	SL	K _{tl}	K _{sl}
N = 3530 for 90 dph and 2136 for 270 dph						
BW	—		0.930**	0.918**	0.202**	0.054**
TL	0.921**		—	0.982**	−0.087	−0.172**
SL	0.921**		0.984**	—	−0.067	−0.268**
K _{tl}	0.185**		−0.023	−0.005	—	0.761**
K _{sl}	0.138**		−0.043 *	−0.065**	0.490**	—
90 dph to 270 dph correlations:						
N = 1630						
		BW	TL	SL	K _{tl}	K _{sl}
		0.398**	0.413**	0.443**	0.256**	0.184**

Notes: The first five rows indicate the inter-trait correlations at 90 dph (above the horizontal) and 270 dph (below the horizontal) in F₂ Asian seabass. BW = body weight, TL = total length, SL = standard length, K_{tl} and K_{sl} = Fulton's condition factor according to total length and standard length respectively. Significance levels: *: $p < 0.05$; **: $p < 0.01$. The last row shows the correlation for each trait between 90 dph and 270 dph. N indicates sample size.

Table 3
Genetic correlation of growth traits in the Asian seabass.

Inter-trait correlations:		BW	TL	SL	K _{tl}	K _{sl}
N = 3871						
BW	—		0.878**	0.876**	0.270**	0.236**
TL	0.942**		—	0.989**	−0.182**	−0.187**
SL	0.946**		0.990**	—	−0.165**	−0.215**
K _{tl}	0.388**		0.189**	0.231**	—	0.934**
K _{sl}	0.284**		0.121**	0.102**	0.886**	—
90 dph to 270 dph correlations:						
N = 3871						
		BW	TL	SL	K _{tl}	K _{sl}
		0.534**	0.430**	0.398**	0.486**	0.363**

Notes: The first five rows show the inter-trait correlations at 90 dph (above the horizontal) and 270 dph (below the horizontal) in F₂ Asian seabass. BW = body weight, TL = total length, SL = standard length, K_{tl} and K_{sl} = Fulton's condition factor according to total length and standard length respectively. Significance levels: *: $p < 0.05$; **: $p < 0.01$. The last row shows the correlation for each trait between 90 dph and 270 dph. N indicates sample size.

is as yet hard to ascertain. Also, for BW, the cross-age genetic correlation is still strong, showing that selecting for BW at a younger age can still be a fairly effective strategy in the F₂ generation. The

lower cross-age phenotypic correlation for BW in F₂ may be explained by environmental changes experienced by the fish as they grow older.

3.4. Estimating heritabilities for growth traits

The number of parents, sires, families and records (each record is the data for one individual) involved in the REML-based estimation of heritability, as well as their parental assignment success rate (PA%) is shown in Table 1. The heritabilities (h^2) based on the various datasets are shown in Table 4. Similar results on the estimation of heritability were obtained using the software Wombat (data not shown). The REML-based estimate of h^2 at 90 dph are: 0.12 ± 0.03 for BW , 0.11 ± 0.03 for TL , 0.10 ± 0.03 for SL , 0.20 ± 0.04 for K_{tl} and 0.11 ± 0.03 for K_{sl} , while those at 270 dph are: 0.34 ± 0.07 for BW , 0.32 ± 0.07 for TL , 0.30 ± 0.06 for SL , 0.13 ± 0.04 for K_{tl} and 0.11 ± 0.04 for K_{sl} . The realised heritability for BW is 0.13. The growth trait estimated heritabilities at 90 dph for F_1 fish are lower than those estimated for the progenitor populations of the same species (Domingos et al., 2013; Wang et al., 2008).

In our study the existence of a larger and phenotypically more varied population enabled us to improve on the precision and certainty of the results in comparison to a previous study in the Asian sea bass (Wang et al., 2008). In that study, which analysed two groups of fish separately, each group did not exceed 900 individuals, and the standard error for each heritability estimate tended to be larger than half of the value of the heritability estimate itself (0.22 ± 0.16 for BW , 0.31 ± 0.14 for TL and 0.22 ± 0.22 for K_{tl} for one group; 0.25 ± 0.18 for BW and 0.24 ± 0.21 for TL and 0.15 ± 0.09 for K_{tl} for the other). In this study, the standard error for every heritability estimated was less than half the value of the heritability estimate itself. Furthermore, the realised heritability for BW at 90 dph is close to the value of the REML-based estimate. Hence we can be fairly confident in the accuracy of our heritability estimates for BW .

The REML-based heritabilities for BW , TL and SL at 270 dph, are higher than those at 90 dph. This is consistent with the study by Domingos et al. in which the heritabilities of Asian seabass wet weight and standard length were higher at harvest age (273 dph) than at 62 dph (Domingos et al., 2013). This data suggests that in terms of BW , a higher accuracy of selection can be achieved at 270 dph than at 90 dph, due to a higher proportion of the phenotypic variation due to additive genetic effects at 270 dph than at 90 dph. In particular, the estimated heritability for BW at 270 dph is the highest among all the estimated heritabilities, indicating that among all the traits considered, BW at 270 dph has the highest accuracy of selection.

A possible reason for the change in heritability of the body size traits with age is indicated by the cross-age correlations for BW , TL and SL , which are noticeably lower than the inter-trait correlations between them. This suggests that different sets of genes controlled body size at 90 dph and 270 dph with some overlap between the two sets but not complete overlap.

In a previous study (Wang et al., 2008) on the progenitor population of Asian seabass, the heritability estimates for BW , TL and K_{tl}

at 90 dph were slightly higher than those estimated in this study. In that study, a systematic decrease in the heritabilities of BW , TL and K_{tl} from F_0 to F_1 was observed. It is known that in breeding programs, truncation selection can reduce the heritability of a trait in a population, due to the fact that brooders have reduced inter-family variation when compared to the overall parental population (Visscher et al., 2008). From F_1 to F_2 , truncation selection had indeed occurred, since the best growers in each batch of F_1 in terms of physical size were selected to be brooders for F_2 . This would have reduced the inter-family variation in body size, which is reflected in the genetic variance component of the phenotypic variance for BW or TL , measures of body size taken in both the earlier and present studies. On the other hand, both generations had been raised in the same facility and under similar growing conditions and likely subjected to similar random environmental effects and hence had similar levels of within-family variation for BW or TL , which is reflected in the other (i.e. residual) variance component of the phenotypic variance of each of those traits. In effect, from F_1 to F_2 , there would have been a decrease in the genetic variance within the phenotypic variances of BW or TL which may explain the decrease in heritabilities reported.

Furthermore, in this study BW , TL and SL were very strongly and positively correlated (r_p , $r_g > 0.8$) with each other in all datasets. Previously, it has been described that heritabilities of directly and indirectly selected traits decrease after selection, and that the change in heritability of the indirectly selected trait depends on its correlation with the directly selected trait (Villanueva & Kennedy, 1990). It is thus reasonable to expect that selection at the age of sexual maturity based on body size would have an effect on the heritabilities of BW , TL and SL at 90 dph.

In this study, we have chosen the REML method to minimize bias due to phenotypic selection. A possible indication that this purpose has indeed been achieved, is that although the realised and REML-based heritabilities for BW at 90 dph were estimated using different sample sizes, resulting from different stages of selection, both estimates were similar. However, a degree of bias is still present, as the pedigree lineage that was used is not fully characterised. It would be ideal to have a truly complete pedigree, but this is difficult when a mass-cross spawning method is used, in which many offspring exist and parentage has to be inferred by genetic markers. Similarly, it would have been ideal to use a complete random sampling of fish at both 90 dph and 270 dph to avoid selection bias. However, in a breeding project where fish are reared in enclosures, it would be impractical to expect a completely random sampling of each generation at 90 dph, since at this age, a significant proportion of those fish that hatched already died due to being unable to compete with their fitter siblings in the limited living space. In other words, those fish that survive at 90 dph have already gone through a non-negligible degree of selection. Hence, bias due to selection is already unavoidable. In the future, advances in genomic kinship reconstruction, as well as in fish rearing technology, may help to eliminate this problem in fish breeding experiments.

In summary, in this study, we reported the growth performance, the inter-trait correlations, the cross-age correlations, as well as the heritabilities for five growth traits on F_2 generation of Asian seabass and compared with the corresponding findings from a previous study on F_1 generation. Interestingly, the cross-age correlations for the same traits between 90 dph and 270 dph are weaker in F_2 than in F_1 , there is a decrease in heritability across the two generations, and the heritabilities at 270 dph are higher than those at 90 dph. Overall, our results show that in Asian seabass F_2 generation, selection at an early age (e.g. 90 dph) can still be effective in producing the desired trait at harvest age (e.g. 270 dph) but there is a possibility that the effectiveness of this method slightly decreases

Table 4
Heritabilities for growth traits in the Asian seabass.

Age		BW	TL	SL	K_{tl}	K_{sl}
90 dph, realised	h^2	0.13	-	-	-	-
REML-based:						
90 dph	h^2	0.12	0.11	0.10	0.20	0.11
	SE	0.03	0.03	0.03	0.04	0.03
270 dph	h^2	0.34	0.32	0.30	0.13	0.11
	SE	0.07	0.07	0.06	0.04	0.04

Notes: F_1 realised and REML-based heritability estimates (h^2) of growth traits of F_1 Asian seabass with standard errors (SE) where applicable. BW = body weight, TL = total length, SL = standard length, K_{tl} and K_{sl} = Fulton's condition factor according to total length and standard length, respectively.

when compared to earlier generations. Further observations and analyses on later generations of fish, may help to ascertain how consistently and rapidly the above-mentioned generational changes in genetic parameters occur in Asian seabass, and how they are related to other parameters such as selection intensity. The results obtained may provide useful information for economic analysis of fish selective breeding programs, such as to estimate the number of generations until no more useful genetic gain can be achieved and the program is no longer profitable.

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