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# Estimated reproductive success of brooders and heritability of growth traits for large yellow croaker (*Larimichthys crocea*) using microsatellites*

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**Abstract** We estimated the reproductive success of adult brood fish and the heritability of growth-related traits for large yellow croaker (*Larimichthys crocea*). We created two independent full-factorial cross groups (1 and 2) by crossing 4 males×4 females and 4 males×3 females, respectively. We measured the body weight (BW), body length (BL), and body height (BH) of 281 individuals from group 1 and 318 individuals from group 2 at 20 months post hatch (harvest age). We also collected a tissue sample from each individual. The parents and offspring were genotyped using six polymorphic microsatellites. Of the 599 offspring, 99.2% were assigned to a single pair of parents. In both groups, some parent pairs produced a large number of offsprings while other pairs did not produce any offspring. The genetic diversity and putative $N_e$ were lower in the offsprings than in the parents in both groups. The heritability estimates at 20-month age were 0.13±0.10 for BW, 0.19±0.13 for BL, and 0.09±0.06 for BH. The genetic and phenotype correlation between BW, BL, and BH was close to 1. The results provide basic information for selective breeding and further genetic characterization of large yellow croaker.

**Keyword**: *Larimichthys crocea*; growth trait; heritability; genetic correlation; microsatellite

## 1 INTRODUCTION

Wild populations of large yellow croaker *Larimichthys crocea* have declined significantly in China since the 1970s because of overfishing, to the extent that they were almost exhaust between the mid 1980s and the late 1990s. To address the decline, researchers began experimenting with the artificial propagation of large yellow croaker in 1985, and were first successful in 1987. By 2005, the annual production of large yellow croaker exceeded 2 billion fingerlings and the harvest was 69 600 tons (Wang et al., 2007). The rapid development of the large yellow croaker aquaculture industry has resulted in a range of issues, including inbreeding depression and germ plasm degradation (Wang et al., 2002). Thus, there is an urgent need to improve the genetic diversity of the cultured stocks.

Understanding the genetic basis of traits is essential for selective breeding programs on improving the performance of a stock. For example, pedigree information is used to estimate the heritability of traits under selection. However, the pedigree of an individual is often difficult to determine as young fish are often too small to tag. Therefore, the pedigree must be reconstructed by genotyping the parents and the offspring using polymorphic DNA markers (e.g. microsatellites). Using this approach, a number of genetic parameters have been estimated in a range of species, including rainbow trout (Fishback et al., 2002), common carp (Vandeputte et al., 2004; Kocour et al., 2007), red drum (Ma et al., 2007; Saillant et al., 2007), silver carp (Gheyas et al., 2009), European sea bass (Dupont-Nivet et al.,

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2008), and Asian sea bass (Wang et al., 2008). However, little is known about the genetic basis of traits (e.g. reproduction and growth) of potential importance to large yellow croaker culture (Wang et al., 2010). We estimated the reproductive success of adult brood fish and the heritability of growth-related traits in harvest age (body weight over 200 g) for large yellow croaker using pedigree information inferred from marker-assisted parentage assignment.

## 2 MATERIAL AND METHOD

### 2.1 Mating design and sampling

The brood fish originated from aquaculture facilities in the following areas: Ningde (26°38′24.95″N, 119°42′26.16″E), Xiapu (26°40′45.54″N, 119°54′14.27″E), Luoyuan (26°21′46.54″N, 119°43′02.06″E), and Lianjiang (26°21′27.34″N, 119°44′30.79″E). These fish were subject to several generations of domestication but were originally derived from wild large yellow croaker that were captured in Guajingyang (26°36′25.23″N, 119°49′16.09″E). Ye (2008) demonstrated that the fish cultured in these regions had very high genetic similarity (>0.88). We created two independent full-factorial cross groups (groups 1 and 2) using large yellow croaker ($n=15$) at a hatchery in Ningde, Fujian Province, China on March, 2007. The first group consisted of 8 adults that originated from Lianjing and Ningde (4 females, 4 males). The second group consisted of 7 adults that originated from Xiapu and Luoyuan (3 females, 4 males). The adult fish were injected with hypothalamic luteinizing hormone-releasing hormone analogue (LRH-A, 3 μg/kg), placed in 1 of 2 tanks (1 tank/group), and allowed to spawn freely. Eggs from the two groups were collected on the second night of spawning and were transferred to one of two 8 000-L incubation tanks. We collected a sample of dorsal fin tissue from each adult after the fertilized eggs were collected. We obtained ~240 000 larvae from group 1 and 180 000 larvae from group 2. The larvae were cultured following standard protocols (Ma, 2005). The fry were reared in a hatchery tank for ~1 month, then transferred to a floating cage in Ningde Sandu'ao Gulf at a mean total length of 3 cm. We observed ~5% mortality during the transfer to the floating cage. Approximately 50 **0**0 fry from each group were reared in two separate cages (54 $m^3$). The size of the cages was increased to accommodate the growth of the fry. The number of individuals decreased from 926/$m^3$ to 18/$m^3$ throughout the experiment. We captured 281 and 318 offspring (20-month-old) from groups 1 and 2, respectively, on October 2008. These fish were weighed to the nearest 0.01 g and measured for body length and body height to the nearest mm. We also collected a fin sample from each fish that was stored in 90% ethanol for genotyping.

### 2.2 Microsatellite analysis and parentage assignment

Genomic DNA was extracted using phenol-chloroform with some modifications to the standard technique. We placed ~50 mg of fin tissue into a 1.5 mL sterile micro-centrifuge tube containing 550 μL TE buffer (100 mmol/L NaCl, 10 mmol/L Tris, pH 8.0, 25 mmol/L EDTA, 0.5% SDS, and 0.1 mg/mL proteinase K, added immediately prior to the tissue). The samples were then incubated for 3.5 h (overnight) at 55°C, and subsequently extracted twice using phenol/chloroform/isoamylalcohol (25:24:1) and chloroform/isoamylalcohol (24:1). The DNA was then precipitated by adding three volumes of precooling ethanol and centrifuging the mixture at 15 000/min (Eppendorf centrifuge 5415D). The pellet was collected and dissolved in 100 μL TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 7.5). We then added 2 μL RNAse (4 μg/μL) and incubated the sample at 37°C for 30 min to eliminate RNA contamination. The DNA was then precipitated by adding three volumes of ice cold ethanol, washed twice with 75% ethanol, air dried, dissolved in TE buffer, and quantified using a spectrophotometer (Beckman DU640). We screened six microsatellite loci that were isolated from an enriched large yellow croaker genomic DNA library for parentage assignment analysis (Table 1.) All primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). PCR amplification was performed in a 20-μL reaction volume containing 40–100 ng template DNA, 1× PCR buffer (10 mmol/L Tris, 50 mmol/L KCl, 15 mmol/L $MgCl_2$, pH 9.0), 200 μmol/L of each dNTP, 0.5 U Taq polymerase (Promega, USA), and 4 pmol of each primer. The thermal cycle consisted of an initial denaturing step at 95°C for 5 min, followed by 30 cycles of 30 s denaturing at 95°C, 30 s annealing at locus-specific temperatures, 30 s extension at 72°C, and a final extension for 10 min at 72°C (Autorisierter Thermocycler, Eppendorf, German). The PCR products were denatured and visualized using denaturing polyacryl-amide gels (6%) followed

**Table 1 Primers, repeat motifs, sequences, and annealing temperature of loci included in the parentage assignment for large yellow croaker**

| Locus | Motif | Primer sequence | Annealing temperature (°C) |
|---|---|---|---|
| LYC0002 | $(TG)_2(AC)_2TC(AC)_{10}$ CTAG$(AC)_5$ | F: 5′-ACCTCCAGTGGGATGTGA-3′ | 50 |
| | | R: 5′-GGCTGTTTGTTATAATTTGTG-3′ | |
| LYC0009 | $(GT)_{10}$TTA$(TG)_4$CTG | F: 5′-GTCAATCACGTCTGTCTCTGC-3′ | 60 |
| | | R: 5′-TCAGCCATTGTCTGTGAGGT-3′ | |
| LYC0011 | $(TG)_{11}$ | F: 5′-CTTTTATTGGCTCCGTATGA-3′ | 55 |
| | | R: 5′-CACTCACACTAGCACGCAC-3′ | |
| LYC0012 | $(AC)_7$ | 5′-CAGAACAAACAATGAATGGG-3′ | 55 |
| | | 5′-GAGGAGCTCAACAGCAACA-3′ | |
| LYC0027 | $(AT)_2(GT)_9G(GA)_2AAT(GA)_2$ TGGTAA$(TGA)_3(TG)_4$ | F: 5′-CACCCAATAATATCGCCATA-3′ | 50 |
| | | R: 5′-GCACACACAATCATCATCATT-3′ | |
| LYC0066 | $(AG)_{10}$ $(GT)_{10}$ T $(TG)_5$ | F: 5′-TTACATGGGCAGCCTGAG-3′ | 56 |
| | | R: 5′-ATGACGCAGCAGAATGG-3′ | |

by silver staining. The parentage analysis was conducted in Cervus 3.0 (Kalinowski et al., 2007) using the NE-PP model (with sex as a known factor).

### 2.3 Statistical analyses

We used a factorial cross design in which females and males had an equal chance of being crossed with each other. Thus, the different families are expected to be equally represented in the progeny for each group. The number of observed progeny per family was compared with a uniform distribution using a chi-square test (SPSS Inc.). The effect of family size was evaluated by calculating the effective genetic size of the population ($N_e$):

$$N_e = \frac{4(N-2)}{\left(K_s + \frac{V_s}{K_s}\right) + \left(K_d + \frac{V_d}{K_d}\right) - 2}$$

where $N$ is the offspring sample size, $K_s$ and $K_d$ represent the mean numbers of offspring per sire and dam, respectively, and $V_s$ and $V_d$ represent the variances in family sizes of sire and dam, respectively (Chevassus, 1989). Under the usual assumptions of random family samples within equally sized families and a large sample of offspring ($n>50$), $N_e$ was also calculated using the classical simplified formula:

$$N_e = \frac{4N_sN_d}{N_s + N_d}$$

where $N_s$ is the number of sires and $N_d$ is the number of dams.

We calculated the allele number ($N_a$), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), polymorphism information contents (PIC) for the brood fish and their offspring using Cervus 3.0 (Kalinowski et al., 2007).

For variance component estimation, the single- and multiple-trait animal models were optimized with MTDFREML (Meyer, 1989, 1991) for estimating heritability as well as additive genetic correlations between traits (multiple-trait model). The animal model is represented by the formula:

$$Y = Xb + Za + e$$

where $Y$ is the vector of (animal) observations (body length, body height and body weight), $b$ is the vector of fixed effects of tank, $a$ is the random vector of additive breeding values, $X$ and $Z$ represent the design matrix for $b$ and $a$, and $e$ is the vector of residuals, or unexplained variance. The very strong linkage between body length, body height, and weight did not permit an adequate convergence of any multi-trait model containing all three variables. Therefore, genetic correlations between body length and weight and between body height and weight were obtained using two-trait models.

## 3 RESULT

### 3.1 Parentage assignment

We used six microsatellite loci for the assignment of parentage (Table 1) A total of 599 individuals were available for parentage assignment, of which 594 could be assigned directly to a single parental

**Table 2 Overall success of parentage assignment for 599 offspring of 7 dams and 8 sires using six microsatellite markers**

| Group | Group 1 | Group 2 |
|---|---|---|
| Assigned | 279 | 315 |
| Unassigned | 2 | 3 |
| Total | 281 | 318 |
| Assignment rate | 99.3% | 99.1% |

pair (Table 2). Five individuals could not be assigned to a single pair as we could not exclude a second parent pair at the 95% confidence level. Thus, 99.2% of the offspring were assigned to a single parental pair and 0.8% could not be assigned.

### 3.2 Differential family representation

The representation of the offspring in the different families is given in Table 3. For group 1, ♀3 and ♂2 produced the highest number of offspring whereas ♀1 and ♂1 produced the least number of offspring. In group 2, ♀5 and ♂6 produced the greatest number of offspring and ♀4 and ♂3 produced the least offspring. The observed numbers of offspring per combination were significantly different from the expected numbers (Group 1: $\chi^2=1\,214.634$, $df=11$, $P<0.001$; Group 2: $\chi^2=940.492$, $df=7$, $P<0.001$). It should be noted that some combinations produced no offspring (e.g. ♀1×♂6 in group 1 and ♀4×♂2 in group 2) and some combinations produced a disproportionate number of offspring (e.g. ♀3×♂2 in group 1 and ♀5×♂6 in group 2).

**Table 3 Number of offspring assigned to parental pairs**

| Group 1 | ♂2 | ♂4 | ♂6 | ♂7 | Sum |
|---|---|---|---|---|---|
| ♀1 | 3 | 1 | 0 | 2 | 6 |
| ♀3 | 177 | 3 | 15 | 2 | 197 |
| ♀5 | 55 | 4 | 5 | 0 | 64 |
| ♀8 | 8 | 4 | 0 | 0 | 12 |
| Sum | 243 | 12 | 20 | 4 | 279 |
| Group 2 | ♂2 | ♂3 | ♂6 | ♂7 | Sum |
| ♀1 | 1 | 1 | 0 | 76 | 78 |
| ♀4 | 0 | 0 | 0 | 2 | 2 |
| ♀5 | 18 | 1 | 208 | 8 | 235 |
| Sum | 19 | 2 | 208 | 86 | 315 |

### 3.3 $N_e$ and diversity of the adults and offspring

The effective population size ($N_e$) was 8 and 6.86 for group 1 and 2, respectively, under the assumption that each adult contributed equally to the total number of offspring. However, based on the actual contribution, $N_e$ was 2.51 and 2.93 (groups 1 and 2, respectively). The loss of putative $N_e$ was 68.6% and 57.3% for groups 1 and 2. The values for $N_a$, $H_o$, $H_e$, PIC for the adults and offspring are presented in Table 4. The genetic diversity was much lower in the offspring than in the parents.

### 3.4 Phenotypic and genetic parameters

The values for body length, body height, and weight are presented in Table 5. The coefficient of variation was highest for weight and lowest for body length. The genetic indices for body length, body height, and weight are presented in Table 6. The estimated heritability for each of these traits was low (range 0.09–0.19). The estimated heritability of body length was highest ($0.19\pm0.13$) whereas the heritability of body height was the lowest ($0.09\pm0.06$). The genetic correlation between these traits was all very high (>0.94). The phenotypic correlations were comparable with the genetic correlations, although the absolute values were lower, and were all significant ($P<0.01$).

**Table 5 Phenotypic indices of large yellow croaker at 20-month age**

| | *N* | Minimum | Maximum | Mean | Std. deviation |
|---|---|---|---|---|---|
| Body length (cm) | 594 | 18.90 | 30.60 | 25.46 | 2.04 |
| Bbody height (cm) | 594 | 5.00 | 9.50 | 7.23 | 0.69 |
| Body weight (g) | 594 | 110.00 | 565.00 | 317.86 | 75.66 |

**Table 4 The allele number ($N_a$), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), and polymorphism information contents (PIC) of adult brood (B) and offspring (O) for 20-month old large yellow croaker**

| | $N_a$ | | $H_o$ | | $H_e$ | | PIC | |
|---|---|---|---|---|---|---|---|---|
| | B | O | B | O | B | O | B | O |
| Group 1 | 5.670 0 | 5.500 0 | 0.875 0 | 0.771 2 | 0.798 6 | 0.663 1 | 0.713 4 | 0.616 0 |
| Group 2 | 5.500 0 | 5.330 0 | 0.809 5 | 0.738 7 | 0.787 5 | 0.671 0 | 0.692 7 | 0.630 2 |

**Table 6 The heritability (along the diagonal), genetic correlation (below the diagonal), and phenotypic correlation (above the diagonal) for growth-related traits in 20-month-old large yellow croaker**

| | BL | BH | BW |
|---|---|---|---|
| Body length (cm) | 0.19±0.13 | 0.78** | 0.91** |
| Bbody height (cm) | 0.95±0.16 | 0.09±0.06 | 0.91** |
| Body weight (g) | 0.99 ± 0.02 | 0.99 ±0.01 | 0.13±0.10 |

** Significantly different $P=0.01$. Values represent the mean±S.E.

## 4 DISCUSSION

Microsatellite markers offer a number of advantages for genetic analyses, including having a high degree of variability, high discriminatory power, and co-dominance. Thus, a number of researchers have used microsatellites for genetic map construction (Ning et al., 2007; Li et al., 2008) and genetic structure analysis (Wu et al., 2009; Zhao et al., 2010) in large yellow croaker. We use six microsatellite markers to conduct a parentage analysis. The efficiency of parentage assignment (>99% for both groups) was very high. However, the microsatellites were genotyped using single locus-PCR, which is time-consuming and costly. Thus, this method is not suitable for large-scale parentage assignment for routine selection. Hence, more cost-effective and rapid multiplex-PCR genotyping systems should be developed. Ideally, all (3–6) markers should be amplified in one PCR reaction and then genotyped (Yue et al., 1999; Neff et al., 2000; Gheyas et al., 2009).

The genetic diversity ($N_a$, $H_o$, $H_e$, PIC) and $N_e$ of the offspring was lower than in the parents because of the unequal contribution of the different crosses. In both groups, some pairs produced a large number of offspring while others produced very few. This is consistent with observations in other species, including salmon (Garant et al., 2001), brown trout (Dannewitz et al., 2004), and Asian sea bass (Wang et al., 2008). The effective population size and genetic diversity of subsequent generations will decrease in instances where some combinations of parents produce few or no offspring. This leads, ultimately, to an increase in inbreeding and reduced selection responses (Wang et al., 2008). These issues may be addressed by increasing the number of brood fish or using artificial fertilization to ensure that each adult has an equal chance of producing offspring.

The precision of heritability estimation is affected by a large number of factors, including the breeding design, the type of relatives, the number and size of the families, the rearing approach, and the method of analysis (Falconer et al., 1996). Our estimates of heritability are lower than other studies (Saillant et al., 2007; Wang et al., 2010) in which the estimated heritability for growth-related traits were all >0.29. It may be due to low number of families that produced >5 offspring. Of the families that produced offspring, only 7 of 13 in groups 1 and 4 of 8 in group 2 exceeded 5 individuals. The standard errors for the heritability of the traits estimated in our study were relatively large, likely because of inequality among the number of progeny from each family. Ideally, the analysis would include equal numbers of fish from each family. However, equalizing family size is difficult due to the unequal reproductive success of brood fish during mass spawning in large yellow croaker. This issue may be resolved to some extent by genotyping more offspring (Wang et al., 2008).

## 5 CONCLUSION

We provide the first estimates for genetic parameters of growth traits in large yellow croaker at harvest age. We demonstrated the practicality of using a factorial mating scheme in a facility that was similar to a small commercial hatchery. Last, we demonstrated that microsatellite markers can be used for pedigree analysis in this species.

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