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Differences in sex reversion and growth between normal and neomale stock in half-smooth tongue sole, *Cynoglossus semilaevis*

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Abstract The present study evaluated the difference in the sex ratio (the genotypic and phenotypic female ratios) and the growth parameters between normal male offspring and neomale offspring under tank culture conditions. The phenotypic female ratio of normal male offspring (20.46–63.79 %) in four families and three stocks was remarkably higher than that of neomale offspring (5.21–10.53 %) in three neomale families and three neomale stocks ($p < 0.01$), while there was no significant difference between normal male offspring (30.41–70.60 %) and neomale offspring (29.7–70.83 %) in the genotypic female ratio ($p > 0.05$). The sex reversion ratio (SRR) was analyzed based on the genotypic and phenotypic female ratios. In neomale offspring, the SRR ranged from 84.25 to 92.53 %, while in normal offspring the SRR ranged from 9.65 to 34.60 %. There was a significant difference between normal male offspring and neomale offspring in the SRR ($p < 0.01$). The weight and length were measured at the ages of 300, 600, and 720 days. The growth rate was analyzed by the statistics, and there was a significant higher growth rate in the normal family than the neomale family ($p < 0.05$). The results suggest that the slower growth in the neomale offspring is responsible for the high SRR.

Keywords *Cynoglossus semilaevis* · Growth difference · Sex reversion difference · Neomale offspring stock · Normal stock

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Abbreviations
SRR Sex reversion ratio
AGRW Absolute growth rate of weight

Introduction

Half-smooth tongue sole *Cynoglossus semilaevis* (Günther, 1873) is a newly exploited, commercially important cultured marine fish in China, and females grow two to four times faster than males (Chen et al. 2008; Fang et al. 2010). The low growth rate of males weakens the quality of the fish and leads to an overall reduction in production. Thus, the production of all female or a high proportion of female stock has been suggested as a means of optimizing *C. semilaevis* production.

In mammalian species, once the sex is determined, it is difficult to reverse. In fish, however, the sex is determined not only by sex chromosomes, but also by the autosome and environmental factors. In certain fish, such as *Oreochromis niloticus* (Linnaeus, 1758), sex change is related to the water temperature (Green and Teichert-Coddington 1993). Therefore, the sex of fish has a considerable plasticity (Devlin and Nagahama 2002; Baroiller et al. 2009). Many fish display the phenomenon of sex reversion. When they are treated with 17a-methyltestosterone, the gonads can revert from ovaries to testes (Basavaraja et al. 1990; Smith and Phelps 2001; Bhandari et al. 2006; Hulak et al. 2008; De Bock and López Greco 2010; Chakraborty et al. 2011). Recently, studies on sex-specific molecular marker screening (Chen et al. 2007), chromosome analysis (Chen et al. 2009), and identification of super-female by SSR female-specific marker (Chen et al. 2012) have been reported for half-smooth tongue sole. Seven female-specific AFLP markers have thus far been identified in tongue sole (Chen et al. 2007). The sex determination system of this species is female heterogametic (ZZ/ZW), and males have ZZ chromosomes, while females have ZW chromosomes (Chen et al. 2007). The neomales are phenotypically male, but revert from being genotypically females (i.e., with the ZW chromosomes). Thus, neomales may be used for mating with females to produce progeny that have a high proportion of genotypic females.

In many fish, the growth ratio was an important index for the breeding of fast-growing species (Gall and Bakar 2002; Janhunen et al. 2013). The Japanese flounder *Paralichthys olivaceus* (Temminck and Schlegel, 1846) was bred as a new fast-growth species as the result of an observation of its growth characteristics. The growth ratio was used to screen for the high-quality families or species (Cheng and Chen 1990).

This study determined the genotypic and phenotypic female ratios and tracked the growth over time of both the neomale offspring and normal stock. This assessment of the growth characteristics and sex ratio of the neomale offspring and normal offspring was performed for two purposes: (1) to analyze the difference in the sex reversion ratio (SRR) between neomale offspring and normal offspring, and (2) to compare the growth characteristics between the normal and neomale offspring.

Materials and methods

Fish

The families and stocks that were utilized in this study were established from breeding stock supplied by the Laizhou Mingbo Aquatic Co., Ltd, and wild stock captured from the

Bohai Sea. The fins of the males were collected for DNA extraction, and the genotypic sex was identified by a female-specific marker (Chen et al. 2007). The neomale was obtained according to Chen et al. (2008) and Deng et al. (2009). The males with the female-specific band were considered to be neomale, while those which did not have the female-specific band were normal males. The mature males numbered 100 individuals with a body weight between 240 and 400 g, and the mature females were 90 individuals between 1,200 and 1,600 g. Twenty of the neomales had a body weight between 350 and 760 g. One male's sperm was used to fertilize one female's eggs, such that we obtained one family. One male's sperm was used to fertilize several females' eggs resulted in the obtaining of a stock. Forty families (30 normal families and 10 neomale families) and 15 stocks (4 normal stocks and 11 neomale stocks) were established in 2008 for analyzing the sex ratio of the families. Ultimately, only 14 normal families, 4 neomale families, 3 normal stocks, and 9 neomale stocks were surivivaled The fins were collected from 14 normal families, 4 neomale families, 3 normal, and 9 neomale offspring stocks and stored in ethanol (Table 1). To identify the phenotypic sex, a portion of the individuals was killed from four random normal families (family 3, 6, 12, and 19), three random neomale families (family 1, 4, and 35) (Table 2), three normal stocks (stock I, II, and III), and three random neomale stocks (stock I, IV, and VI) (Table 3), and the gonads were fixed in Bouin's fixative to assess the phenotypic sex by microscopic inspection of histological sections of the gonads. With the same temperature and stocking density as the 2008 year, 70 families (40 normal families and 30 neomale families) were established in 2010 in order to evaluate the growth characteristic, and only 25 normal families and 14 neomale families were survivaled. Ultimately, 12 normal families and 8 neomale families were randomly selected to evaluate the growth characteristic. All of the fish in year 2008 and 2010 was cultured in the same temperature (22 °C), stocking density (fry 250–300/m^2), feeding regimes (3 times day^{-1}), dissolved oxygen (above 5 mg L^{-1}), and seawater (salinity 15–30 g kg^{-1}). To analyze the growth characteristics of the neomale families and normal families, 30 individuals were selected from several random normal families (family 6, 28, 38, 39, and 61) to establish a single stock (control). The body weight and body length of 100 individuals from the various families were measured at the ages of 300, 600, and 720 days (Table 4).

DNA extraction from tissue

About 10–30 mg sample of fin tissue was homogenized in 500 μL lysis buffer (10 mM L^{-1} Tris–HCl, pH 8.0; 100 mM L^{-1} EDTA, pH 8.0; 100 mM L^{-1} NaCl; 0.5 % SDS, and freshly added proteinase K 100 μg mL^{-1}). Then, the homogenate was lysed at 55 °C for 1 h. DNA was extracted via the phenol–chloroform method. DNA was precipitated with two volumes of ethanol. The DNA was first washed with 70 % ethanol and then put in absolute ethanol, dried, and then dissolved in sterile water. The quality and

Table 1 Number of the families and stocks used in the study

	Normal family	Neomale family	Normal stocks	Neomale stocks	Utilizations
2008	14	4	3	9	Identify the genotypic sex
	4/14	3/4	3/3	3/9	Identify the phenotypic sex
2010	12/25	8/14			Evaluate the growth ratio

4/14: 4 Random families from 14 normal families

concentration of DNA were assessed by agarose gel electrophoresis and were measured with a GENEQUANT Pro (Pharmacia Biotech Ltd.) RNA/DNA spectrophotometer.

Genotypic sex identification using female-specific AFLP markers

Based on the sequences of the female-specific AFLP fragments, a pair of specific SCAR primers (CseF382F 5′ATTCACTGACCCCTGAGAGC3′ and CseF382R 5′TGGCACCAT CATTGTAAAACTA3′) were designed for genotypic sex identification. The PCR system (15 μL) consisted of 0.5 μL of 10 pM of each primer, 1.2 μL of 2.5 mM of each dNTP, 0.2 μL of Taq polymerase (Takara, Bio., Dalian, China), 1.5 μL of 10× Taq buffer (Mg^{2+} Plus), and 1 μL of sole DNA as the template. The PCR was run as follows: incubation at 94 °C for 4 min, followed by 32 cycles of 94 °C, 30 s; 57 °C, 30 s; and 72 °C, 40 s, with a final extension of 6 min at 72 °C. The amplification products of 15 μL were resolved on 1.5 % agarose gel with a DL2000 DNA marker. A female-specific fragment of 291 bp was amplified from the female genome.

Statistical analysis

The sex ratios between normal families and neomale offspring families were determined by the independent *t* test. All of the means are reported ± SE. Differences were considered significant when $p < 0.05$ (Sokal and Rohlf 1981). Sex reversion ratio was analyzed by SRR = (P1 − P2)/P1 (where SRR is the sex reversion ratio, P1 the genotypic female ratio, and P2 phenotypic female ratio). The absolute growth rate was analyzed by AGRW (g day^{-1}) = (W2 − W1)/(T2 − T1). (T1 and T2 are the age in days; W1 is the weight at T1, and W2 is the weight at T2). The growth data in the various families were analyzed by one-way ANOVA followed by Duncan multiple comparison tests using SPSS 17.0 (IBM, New York). Significance was set at $p < 0.05$. The data of weight and AGRW were analyzed between normal stock (all of the normal families) and neomale stock (all of the neomale families) by independent *t* test.

Results

Genotypic and phenotypic sex identification of normal and neomale families

The genotypic sex was identified for 14 normal families and 4 neomale families, with the highest genotypic female ratio being 70.60 % (family 6), and the lowest 30.41 % (family 19) in the 14 normal families (Table 2). In the four neomale families, the lowest female ratio was 29.70 % and the highest was 66.70 %. Statistical analysis revealed that there was no significant difference between the normal families and neomale families ($p > 0.05$). Phenotypic sex analysis of the families revealed that the phenotypic female ratio ranged from 20.46 to 63.79 % in the four normal families and from 5.88 to 10.53 % in the three neomale families. There was a significant difference between the normal and neomale families ($p < 0.01$). The SRR ranged from 9.65 to 34.60 % in the normal families and ranged from 84.25 to 90.00 % in the neomale families. There was a significant difference in the SRR between neomale families and normal families ($p < 0.01$).

Genotypic and phenotypic sex identification of normal and neomale offspring stocks

In terms of the identification of the genotypic and phenotypic sex for the three normal and nine neomale stocks, the genotypic female ratios ranged from 46.88 to 51.04 % in the three normal stocks and from 43.18 to 70.83 % in the nine neomale stocks (Table 3). No significant difference was found between the normal and neomale stocks in terms of the genotypic female ratios ($p > 0.05$). The phenotypic female ratio ranged from 40.63 to 42.71 % in the normal stock. However, the phenotypic female ratio in the neomale stock ranged only from 5.21 to 6.25 %. Statistical analysis revealed that the phenotypic female ratio did not deviate from 50 % ($p > 0.05$), while in the neomale stock there was a significant decrease in the phenotypic female ratio ($p < 0.01$) and a significant deviation from 50 % ($p < 0.01$). The SRR ranged from 12.77 to 16.33 % in the three normal stocks and ranged from 91.18 to 92.53 % in the three neomale stocks. A significantly different SRR was found in the neomale stock compared to the normal stock ($p < 0.01$) (Table 3).

The differences in the growth characteristics between the normal and neomale

The body weight and body length of the fish in the 12 normal and 8 neomale offspring families were measured at the approximate ages of 300, 600, and 720 days (Table 4), respectively. At the age of 300 days, the average body length of the normal families was 12.20 ± 2.24 cm and the average body weight was 12.08 ± 5.69 g; however, the average body length of the neomale families was 13.83 ± 1.92 cm and the body weight was 16.79 ± 5.70 g. A significantly faster growth ratio was found in the neomale families than in the normal families ($p < 0.05$). At the ages of 600 and 720 days, the average body length of normal families was 27.2 ± 5.3 and 33.4 ± 6.9 cm, and the body weight was 138.0 ± 70.6 and 257.4 ± 171.95 g, respectively. The average body length of neomale families was 24.8 ± 2.77 and 28.8 ± 4.8 cm and the body weight was 101 ± 41.41 and 157.3 ± 105.97 g. The significantly faster growth ratio was found in the normal families than in the neomale families ($p < 0.05$). The absolute growth rate ranged from 0.26 to 0.44 in the 12 normal families and ranged from 0.16 to 0.35 in the 8 neomale families. In 12 normal families, only one family's (family 5) absolute growth rate was lower than the control family, and in 8 neomale families, only one family's (family 60) absolute growth rate was higher than the control family. The result of statistic analysis showed that there was a significant difference between neomale families and normal families in AGRW ($p < 0.05$).

Discussion

Teleost fishes are the most abundant vertebrates and have an unmatched diversity of species. Not surprisingly, given this extreme diversity, these fish exhibit all of the known forms of vertebrate sex determination. The most common mode of sex determination appears to be genotypic sex determination (GSD), where the sex is determined by the inherited combination of sex-determining genes and "minor" genotypic factors (i.e., polygenic systems) (Devlin and Nagahama 2002; Oldfield 2005). However, various forms of environmental sex determination (ESD) have also been reported. For example, environmental factors such as water temperature and pH are known to influence sex determination in certain species (Godwin et al. 2003; Oldfield 2005; Ospina-Álvarez and Piferrer 2008). Temperature-dependent sex determination (TSD) is the most common form

Table 2 Genotypic and phenotypic sex determination, and the SRR in 14 normal families and 4 neomale families

Family no.	No. of genotype female	No. of phenotype female	Total	Genotypic female proportion (%)	Phenotypic female proportion (%)	SRR (%)
Normal family (2008)						
2	45	–	85	52.90	–	–
3	43	31	92	46.74	33.70	27.89
6	41	37	58	70.60	63.79	9.65
7	41	–	73	56.16	–	–
10	42	–	87	48.30	–	–
12	52	34	109	47.71	31.20	34.60
14	30	–	64	46.87	–	–
15	20	–	36	55.60	–	–
16	32	–	60	53.30	–	–
19	52	35	171	30.41	20.46	32.72
27	26	–	62	41.94	–	–
28	26	–	68	38.30	–	–
29	34	–	58	58.60	–	–
30	32	–	70	45.71	–	–
Mean ± SD				49.51 ± 9.32	37.28 ± 13.25	26.21 ± 11.39
Neomale family (2008)						
1	76	12	114	66.70	10.53	84.25
4	50	5	82	60.98	6.10	90.00
34	16	–	54	29.70	–	–
35	28	3	51	54.90	5.88	89.29
Mean ± SD				53.07 ± 14.12	7.50 ± 2.62**	87.84 ± 3.13**

SRR sex reversion ratio, – means the data were not collected

** Indicates the difference is significant at the 0.01 level ($p < 0.01$)

Table 3 Genotypic and phenotypic sex determination, and the SRR in three normal and nine neomale stocks

Population no.	No. of genotype females	No. of phenotype females	Total	Genotypic female proportion (%)	Phenotypic female proportion (%)	SRR (%)
Normal population (2008)						
i	45	39	96	46.88	40.63	13.33
ii	47	41	96	48.96	42.71	12.77
iii	49	41	96	51.04	42.71	16.33
Mean ± SD				48.96 ± 2.08	42.01 ± 0.98	14.14 ± 1.91
Neomale population (2008)						
I	67	5	96	69.79	5.21	92.53
II	26	–	53	49.05	–	–
III	42	–	77	54.55	–	–
IV	66	5	96	68.75	5.21	92.42
V	34	–	55	61.82	–	–
VI	68	6	96	70.83	6.25	91.18
VII	22	–	48	45.83	–	–
VIII	21	–	48	43.75	–	–
IX	19	–	44	43.18	–	–
Mean ± SD				56.39 ± 10.92	5.56 ± 0.60**	92.04 ± 0.61**

SRR sex reversion ratio, – means the data were not collected

** Indicates the difference is significant at the 0.01 level ($p < 0.01$)

Table 4 Comparison of growth in different families (year of 2010) of half-smooth tongue sole

Type of family	No. of family	Age of 300 days		Age of 600 days		Age of 720 days		AGRW (g day^{-1})
		Body length (cm)	Body weight (g)	Body length (cm)	Body weight (g)	Body length (cm)	Body weight (g)	
Normal	5	15.57 ± 2.04^{jk}	21.57 ± 8.88^{h}	26.77 ± 5.36^{bcd}	141.73 ± 107.17^{bcdef}	30 ± 6^{bc}	192.4 ± 159.3^{abcdef}	0.26
Family	6	15.95 ± 2.23^{k}	24.15 ± 11.25^{i}	28.29 ± 4.29^{def}	152.49 ± 93.10^{def}	32.7 ± 5.5^{cdef}	241.6 ± 165.2^{defghij}	0.33
	21	14.61 ± 1.69^{hi}	18.30 ± 6.45^{g}	29.35 ± 3.63^{fg}	164.27 ± 65.64^{ef}	32.1 ± 4.2^{cdef}	212.3 ± 114.6^{cdefgh}	0.29
	28	12.21 ± 2.51^{f}	11.76 ± 6.50^{cde}	28.82 ± 5.38^{efg}	168.76 ± 104.95^{f}	34.7 ± 7.5^{fg}	292.8 ± 199.5^{hij}	0.41
	38	11.38 ± 1.94^{de}	9.52 ± 3.83^{bc}	27.83 ± 5.17^{cdef}	140.78 ± 75.11^{bcdef}	34.4 ± 7.6^{efg}	281.7 ± 160.3^{ghij}	0.39
	39	13.26 ± 1.99^{g}	13.12 ± 5.26^{def}	27.68 ± 5.53^{cdef}	146.16 ± 86.99^{bcdef}	31.1 ± 7.6^{bcdefg}	200.8 ± 160.6^{bcdefg}	0.28
	40	13.03 ± 1.83^{g}	14.63 ± 5.28^{f}	26.40 ± 5.77^{abcd}	148.86 ± 102.10^{def}	31.4 ± 8.2^{bcdef}	240.1 ± 189.2^{defghij}	0.33
	44	13.36 ± 2.19^{g}	13.98 ± 6.08^{ef}	27.61 ± 5.10^{cdef}	146.80 ± 93.06^{cdef}	32.1 ± 5.4^{cdef}	229.6 ± 140.9^{cdefghi}	0.31
	57	11.38 ± 1.55^{de}	9.10 ± 3.19^{bc}	27.57 ± 5.61^{cdef}	152.83 ± 99.47^{def}	33.3 ± 8.4^{cdef}	272.1 ± 208.2^{fghij}	0.38
	61	10.41 ± 1.30^{c}	8.05 ± 12.03^{b}	30.40 ± 7.9^{g}	171.98 ± 89.8^{f}	37.0 ± 7.2^{g}	320.1 ± 171.1^{j}	0.44
	63	7.09 ± 1.34^{a}	2.99 ± 1.22^{a}	27.21 ± 4.90^{cde}	148.12 ± 89.72^{cdef}	33.5 ± 7.6^{def}	302.3 ± 207.8^{ij}	0.42
	69	9.02 ± 0.98^{b}	3.35 ± 1.19^{a}	27.20 ± 4.64^{cde}	128.76 ± 75.42^{bcd}	32.7 ± 7.1^{cdefgh}	211.4 ± 134.9^{cdefgh}	0.30
	Control	11.93 ± 2.08^{ef}	10.25 ± 5.10^{bc}	26.27 ± 4.74^{abc}	128.27 ± 79.36^{bcd}	30.5 ± 6.2^{bcd}	198.3 ± 153.9^{abcdef}	0.27
	Mean ± SD	12.20 ± 2.24	12.08 ± 5.69	27.2 ± 5.3	138.0 ± 70.6	33.4 ± 6.9	257.4 ± 171.95	0.35 ± 0.23
Neomale	3	16.90 ± 2.07^{l}	26.93 ± 11.94^{j}	26.87 ± 3.82^{bcd}	129.88 ± 51.55^{bcd}	30.4 ± 3.4^{bcd}	170.2 ± 73.5^{abcde}	0.23
Family	4	14.88 ± 1.56^{ij}	17.07 ± 6.07^{g}	26.47 ± 2.30^{abcd}	114.75 ± 28.47^{ab}	29.9 ± 3.4^{bc}	155.5 ± 50.2^{abc}	0.21
	7	15.54 ± 1.74^{jk}	23.17 ± 7.29^{hi}	27.09 ± 3.73^{cde}	132.98 ± 73.22^{bcde}	30.6 ± 4.4^{bcd}	162.4 ± 62.5^{abcd}	0.23
	14	14.04 ± 2.53^{h}	17.06 ± 9.10^{g}	27.60 ± 3.92^{cdef}	150.58 ± 77.04^{def}	31.3 ± 6.3^{bcde}	222.4 ± 178.4^{cdefghi}	0.31
	17	14.33 ± 1.56^{hi}	18.07 ± 5.76^{g}	27.53 ± 4.28^{cdef}	146.16 ± 70.06^{bcdef}	30.8 ± 5.7^{bcd}	197.6 ± 125.0^{abcdef}	0.27
	20	11.27 ± 1.87^{f}	10.73 ± 3.86^{bcd}	24.92 ± 2.66^{a}	95.29 ± 31.37^{a}	26.5 ± 2.5^{a}	117.8 ± 45.9^{a}	0.16
	56	12.63 ± 1.96^{fg}	11.66 ± 5.05^{cde}	24.86 ± 2.80^{a}	95.69 ± 34.15^{a}	28.3 ± 3.3^{ab}	124.0 ± 36.7^{ab}	0.17

Table 4 continued

Type of family	No. of family	Age of 300 days		Age of 600 days		Age of 720 days		AGRW (g day^{-1})
		Body length (cm)	Body weight (g)	Body length (cm)	Body weight (g)	Body length (cm)	Body weight (g)	
	60	11.05 ± 2.23^{cd}	9.60 ± 4.23^{bc}	25.26 ± 4.13^{ab}	116.29 ± 69.49^{abc}	32.6 ± 6.8^{cdef}	252.8 ± 187.2^{efghij}	0.35
	Mean ± SD	13.83 ± 1.92	16.79 ± 5.70*	24.8 ± 2.77	101 ± 41.41*	28.8 ± 4.8	157.3 ± 105.97*	0.21 ± 0.14*

Each weight value is the average ± SEM ($n = 100$); The data were analyzed by one-way ANOVA followed by Duncan comparison tests using SPSS 17.0

Different letters differed with statistical significance ($p < 0.05$). * Indicates the difference is significant at the level ($p < 0.05$)

of ESD in vertebrates and has been reported in over 50 fish to date (Valenzuela et al. 2003; Conover 2004; Hattori et al. 2009). TSD occurs when the temperature during a critical period of development either determines the direction of sex differentiation or acts in combination with other sex determination mechanisms (e.g., genotype by genotype affected by environment interaction) to ultimately influence the phenotypic sex of the animal. This is the case in the half-smooth tongue sole when it was treated with high temperature or methyltesterone (MT) from 30 to 100 days old, the female (ZW) reverted to male (ZW) (Chen et al. 2008), which was called neomale. When the neomale mated with a female, the ZW combines with the other ZW chromosomes, which results in 25 % ZZ, 25 %WW, and 50 % ZW offspring. Therefore, the neomale offspring are approximately 75 % female. Because of the presence of a lethal locus on the W chromosome close to the sex-determining locus, there is a high mortality of homogeneous recessive alleles, as suggested by Komen et al. (1991) in other fishes. If it is the case that the lethal locus is more distant from the centromere than the sex-determining locus, then the putative WW females with the heterozygous lethal loci would not die if a single crossover occurred between the site. Furthermore, high recombination rates have been observed in half-smooth tongue sole (Chen et al. 2009), and WW super-female only determined in the embryo, not in fry (Chen et al. 2012). Therefore, a female with a ZW or WW genotype would have the capacity to produce totally different sex ratios and would thus exhibit less of a lethal effect. Thus, the female ratio would be expected to be 66.67–75.00 %. In fact, however, we detected a genotypic female ratio of 1:1 in neomale families. The genotypic female ratio was not between 66.67 and 75.00 %, but rather approximately 50 %. The same result that genotypic female ratio of 1:1 in neomale families was detected in neomale family by female-specific SSR maker (data no show). The inactive W gamete of neomale may contribute to the genotypic female ratio of 1:1 in neomale family (Chen et al. 2014).

Simultaneously, the phenotypic female ratio was tested by inspection of histological sections of the gonads. There was a significant difference between the neomale offspring and normal offspring ($p < 0.01$). The phenotypic female ratio in these two populations deviated from the 50 % that is a universal phenomenon in fish as a result of environmental control of sex differentiation and autosome influence (Yamamoto and Kajishima 1968; Nomura et al. 1998). Corresponding to the genotypic female ratio, we found that most of the genotypic female reversed to the phenotypic male.

The SRR was significantly higher in the neomale families and stocks compared to the normal families and stocks ($p < 0.01$). This is an interesting phenomenon in fish. Recently, in European sea bass, it was reported that DNA methylation plays an important role in the sex ratio shift (Navarro-MartÍn et al. 2011). In addition, genome-wide methylation is reported to have an important influence on sex (Liu et al. 2010). Recently, the genome-wide mapping of methylation showed us that the degree of methylation in neomale was higher than in normal male or female (Shao et al. 2014). Thus, we infer that the high SRR may be related to DNA methylation, but further study is needed.

The growth characteristics were analyzed between neomale families and normal families. There was a significant difference between normal families and neomale families in the growth rate. At the age of 300 days, a significantly faster growth ratio was found in the neomale families than in the normal families ($p < 0.05$); however, at the ages of 600 and 720 days, a significantly faster growth ratio was found in the normal offspring compared to the neomale offspring ($p < 0.05$). From the statistical analysis of the total weight and total length between the various families, the result shows that at the age of 300 days, several families growth rate (5, 6, 21, 39, 40, 44, 4, 7, 14, 17) was significantly higher than the control families, and some families growth rate (63, 69) was significantly lower than the

control families ($p < 0.05$); at the age of 600 days, the growth rate of families 21, 28, and 61 was significantly higher than control families and the growth rate of families 20 and 56 was significantly lower than the control family ($p < 0.05$); at the age of 720 days, the growth rate of families 28, 38, 57, 61, and 63 was significantly higher than control family ($p < 0.05$). According to the sex reversal ratio of the neomale offspring and normal offspring, high sex reversal ratio in neomale offspring resulted in more phenotypic male in neomale families. Zhang (2010) reported that half-smooth tongue sole grew faster at 27 °C than any other temperatures at the age of 1 year. Besides, Deng et al. (2007) reported that high temperatures (28 °C) produced a higher proportion of phenotypic males. Thus, we speculated that the male half-smooth tongue sole grew faster that female before the age of 300 days. So the neomale families grew faster than normal families before age of 300 days, and after age of 300 days, the normal families grew faster than neomale families.

The absolute growth rate was also analyzed and there was a significantly higher AGRW in normal families than in neomale families ($p < 0.05$). The result showed that normal families grew faster than neomale families. The low growth rate in neomale families attributed to the high sex reversal ratio and more genetic female reverse to phenotypic male. Besides, the result of absolute growth rate among various families showed us that there was a significant difference. The AGRW of some families (6, 14, 21, 28, 38, 39, 40, 44, 57, 60, 61, 63, 69) was higher than the control family (0.27) and the AGRW of several families (3, 4, 5, 7, 20, 56) was lower than the control family (0.27). These suggested that fastest growth rate family could be selected by family selection.

In summary, to detect the sex ratio of neomale and normal offspring, the genotypic and phenotypic sex were detected in the neomale and normal populations. A significantly higher SRR was found in the neomale population compared to the normal population. Thus, the weight and length were measured in the normal and neomale families. We found that most of the neomale families grew more slowly than the normal families and the difference was significant ($p < 0.05$). The slow growth rate was responsible for the high SRR in the neomale offspring. Most of the genotypic female reversed to a phenotype male, and males grew more slowly than females in the same environment. Thus, neomale offspring grew more slowly than normal families or stocks. These finding lay the foundation and provide important way for breeding high female and grow faster half-smooth tongue sole in commercial culture.

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