

Research Article

Growth Patterns and Growth-Axis Gene Expressions in Sexual Dimorphism of *Silurus asotus* Linnaeus, 1758

Zhigang Qiao ¹, Muzi Li,¹ Miao Yu,¹ Meng Zhang,¹ Lei Wang,¹ Hongxia Jiang,¹ and Sijia Liu ²

¹College of Fisheries, Henan Normal University, Xinxiang, Henan, China

²Qinghai Provincial Key Laboratory of Animal Ecological Genomics, Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, Qinghai, China

Correspondence should be addressed to Zhigang Qiao; 13503800008@126.com and Sijia Liu; liusj@nwipb.cas.cn

Received 5 November 2022; Revised 12 December 2022; Accepted 17 January 2023; Published 30 January 2023

Academic Editor: Mohamed Abdelsalam

Copyright © 2023 Zhigang Qiao et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Aquaculture has tremendous economic significance in distinguishing males and females in the juvenile *Silurus asotus* (Linnaeus, 1758) to obtain a female population with tremendous growth potential. To investigate the potential biological markers between young males and females *S. asotus*, we analyzed the characteristics of sexual dimorphism by measuring the 14 length traits and 9 weight indicators in an artificial insemination population at 3, 5, and 7 months. In addition, quantitative real-time PCR (qRT-PCR) was performed to determine the sexually dimorphic expression of the growth hormone-1 gene (*GH-1*), growth hormone receptor gene (*GHR*), and insulin-like growth factor gene (*IGF-1*) in the hypothalamus, pituitary, gonad, and liver, at 3, 5, and 7 months. The results showed that in morphology, except for eye diameter and the distance between the pelvic and anal fins in 3-month fish, all other morphological indicators were significantly ($P < 0.05$) or very significantly ($P < 0.01$) different between juvenile males and females. The visceral weight, eviscerated weight, and intestine weight in females were significantly ($P < 0.05$) or very significantly ($P < 0.01$) higher than in males at 5 and 7 months. Joint static allometric analyses on 14 length indicators relative to weight showed different sex growth patterns in 3-month, 5-month, and 7-month fish. In gene expression patterns, *GH-1*, *IGF-1*, and *GHR* were highly expressed in the pituitary, with higher levels in females ($P < 0.05$ or $P < 0.01$). In contrast, the three genes were all more highly expressed in the testis than in the ovary ($P < 0.01$), indicating their essential roles in testis development. Our results demonstrate that *S. asotus* has female-biased sexual dimorphism. The length traits related to head shapes could be the potential phenotype marker to distinguish females and males in 7-month juveniles.

1. Introduction

Silurus asotus (Linnaeus, 1758) belongs to the order Siluriformes, the family Siluridae, and the genus *Silurus*, which is distributed throughout the vast watershed of rivers in China [1]. *S. asotus* is an essential freshwater commercial fish with tender meat, relatively few bones, and abundant nutrients, which is therefore favored by consumers [2]. In China, the annual yield of *S. asotus* amounts to 315,322 tons, which produces an output of 312,169 USD (<https://www.fishbase.se/report/FAO/>, updated in 2007). Biologically, *S. asotus* takes three to four years to be sexually mature, and females have a larger size and more weight than males, with faster

growth performance [1]. Thus, generating an all-female population in the juvenile *S. asotus* could create more economic benefits in aquaculture [3]. However, it is difficult to tell the sexes apart with the naked eye when the fish are young. Although the study of genetic sex determination has been carried out and has found several potential molecular markers, no genetic marker has been proven in production so far. Several studies have demonstrated that some fish exhibit allometric growth characteristics in whole or part between females and males [4], which provide potential phenotypic traits to distinguish the sexes in young fish. However, studies on the sexually dimorphic growth patterns in juvenile *S. asotus* are still scarce.

Sexual dimorphism is the systematic difference in size, shape, color, physiology, and behavior between male and female individuals of the same species [5], which shows universality and diversity in teleosts [6]. The sexual selection theory asserts that sexual dimorphism results from sexual selection, but the causes of sexual dimorphism differ among fish species [7]. For the male-male competition, males are larger than females, such as *Ictalurus punctatus* [8], *Odontobutis obscura* [9], *Oreochromis niloticus* [10], *Pelteobagrus fulvidraco* [11, 12], and *Abbottina rivularis* [13]. Selection for fecundity, however, which results in females being larger than males [14], is the cause of sexual dimorphism in species such as, *Salmo gairdneri* [15], *Dicentrarchus labrax* [4], *Acrossocheilus wenchowensis* [16], *Paralichthys olivaceus* [17], *Cynoglossus semilaevis* [18] and *Gambusia affinis* [19]. In addition, differences in early growth rates between the two sexes may also result in sexual dimorphism [20]. In *D. labrax*, several sex-related growth patterns occur at early development stages [21].

Allometric scaling describes the relative growth relationship between different local body sizes or between whole and partial body sizes. The differentiation in allometries among traits has been thought to be a driving force by which morphology and structure evolve [22]. Huxley and Teissier [23] proposed the initial equation of allometry to quantify allometric scaling. In the simple allometry equation, $y = \alpha \cdot x^b$ (where b is the allometric scaling), the two variables could be the length, weight, shape, density, and volume [24, 25]. An advanced allometry equation named the joint static allometric scaling model [26] was proposed to simultaneously evaluate the allometry scalings of multiple body parts to the entire body. This equation is currently the most common method used in allometric studies of commercial fishes, such as *P. olivaceus* [27] and *O. niloticus* [28].

Some studies have indicated that the phenotypes of sexual dimorphism in invertebrates are the consequences of sex-biased gene expression and are controlled by multiple critical genes during growth and development [29]. The growth, reproduction, and metabolism of fish mainly depend on the somatotrophic axis (brain-pituitary-liver) and reproductive axis (brain-pituitary-gonad) [30]. Comparative transcriptome analysis revealed many differentially expressed genes (DEGs) in the hypothalamus and pituitary of *Mystus wyckioides* between males and females, and most of these DEGs were involved in gonad development and growth [31]. Shen et al. [1] identified many sex-specific DEGs in the ovary and testis of *S. asotus*. These DEGs were enriched in calcium signaling and cytokine-cytokine receptor interaction for males and in RNA transport and ribosome biogenesis for females [1]. Sex-specific effects of the growth hormone gene (*GH*) and its downstream effector, the insulin-like growth factor-1/2 gene (*IGF-1/2*), play critical roles in the growth difference between males and females [32]. In teleosts, somatic growth is greatly regulated by the *GH/IGF-1/2* axis genes expressed in the hypothalamus-pituitary-gonad (HPG) axis [33]. The expressions of *GH*, *IGF-1*, and *IGF-2* were significantly higher in males than

in females in both larval and adult *P. fulvidraco*, which are involved in sexual size dimorphism [34]. *IGF-1* is a mediator of growth hormone (*GH*), as has been reported for humans, mice, and several fish species [35–37]; *IGF-2*, in general, acts more independently from *GH* and seems to be crucial for early embryonic growth, as has been demonstrated in mouse embryos [37]. In humans, *IGF-1* levels are associated with body mass index and fat deposits in adult women [38]. In addition, the *IGF-1* gene is widely used in agriculture as a potential biomarker [39, 40].

Considering the importance of basic biological studies for freshwater fisheries, this study detected the sexually dimorphic morphology, growth patterns, and expression levels of brain-pituitary-gonad/liver genes in *S. asotus* at different developmental stages. The study could accumulate basic data for the implementation of scientifically based breeding and increase the overall production of *S. asotus* farming.

2. Materials and Methods

2.1. Experimental System and Fish. The juvenile *S. asotus* were obtained by artificial breeding, according to Mao et al. [41]. They were raised in the freshwater fish breeding base of Henan Normal University (Qi County, Henan, China). The experiment was carried out at the breeding base. A total of 900 juvenile fish, aged one month, were randomly collected by a dense fishing net (mesh size was 1 mm) and kept in 9 identical 300 L tanks (with a density of 0.3 fish/L) filled with circulating fresh water in the breeding base. Each tank was equipped with continuous aeration and was maintained at a 12 : 12 L : D light-dark regime. Fish were fed (3 times a day 08:00, 13:00, and 18:00) with commercial pellets with 43% protein (Hanye Biotechnology Co., Ltd., China). The pH and the water temperature are 7.8–8.0 and 20–23°C, respectively. Uneaten food and fish feces were removed daily. Freshwater with the desired temperature was exchanged at a rate of 40% twice a day to minimize the build-up of nitrates and nitrites and maintain water quality. At 3, 5, and 7 months, respectively, 30 juvenile *S. asotus* from each tank were randomly collected and anesthetized with a lethal dose (500 mg/L) of MS-222 (Zhonghong Biological Engineering Co., Ltd., China) to measure the length traits, the weight indicators, and mRNA extraction.

2.2. Length Traits Detection. The 14 length traits, including body length, body depth, body width, head depth, head length, head width, snout length, postorbital head length, eye diameter, interorbital width, caudal peduncle depth, caudal fin length, pelvic fin precoxal length, and distance between the pelvic and anal fins, were measured by a ruler and a vernier caliper (accurate to 0.1 mm), respectively.

2.3. Weight Traits Detection. After the body weight was measured, the fish was dissected on ice. Weight traits, including visceral weight, eviscerated weight, fat weight, gonad weight, intestine weight, kidney weight, liver weight, and spleen weight, were measured. Meanwhile, the sex was

determined by gonad evidence. The individual whose gender could not be determined was discarded.

2.4. Coefficient of Maturity and Coefficient of Fatness. The coefficient of maturity and the coefficient of fatness were calculated. The formulas are as follows:

$$\text{Maturity} = \text{gonad weight (g)} / \text{body weight (g)} \times 100 \quad [42]$$

$$\text{Condition factors} = \text{body weight (g)} / \text{body length (cm)}^3 \times 100 \quad [42]$$

2.5. Allometric Scaling of Length Traits to Body Weight. The joint static allometric scaling model [26] calculated the allometric scaling scale. The initial model is as follows: $y = b_0 \cdot x_1^{b_1} \cdot x_2^{b_2} \dots x_m^{b_m}$, where x_1, x_2, \dots, x_m indicate partial body size m ; b_0 is the normalized constant; and b_1, b_2, \dots, b_m is the biased allometric scaling of the i ($i = 1 \dots m$) partial body size to the whole body size. The model was converted by taking the natural logarithm to facilitate the calculation. Then, the equation is converted as follows: $\ln y = \ln b_0 + b_1 \ln x_1 + b_2 \ln x_2 + \dots + b_m \ln x_m$. Using the converted equation, a stepwise regression was performed to obtain the optimal static allometric scaling model.

2.6. qRT-PCR. Determination of the mRNA expression levels was performed by quantitative real-time PCR (qRT-PCR) in a LightCycler® 480 thermocycler (Roche, Germany) in a total volume of 20 μ l with the LightCycler® 480 SYBR Green I Master (Roche, Germany) following the manufacturer's protocol. The qRT-PCR experiment was performed at 95°C for 5 min, 45 cycles (95°C for 15 s), and 60°C for 45 s. The relative expression of target genes was calculated using the $2^{-\Delta\Delta C_t}$ method. All amplification reactions were carried out in triplicate, and a nontemplate control was also included in each run. The relative expression level of mRNA was normalized with the β -actin gene and calculated using the comparative threshold cycle method [43]. Primers (Table 1) for qRT-PCR were designed using the Sangon Biotech web server (<https://www.sangon.com/>) and Oligo7 software [44] based on the ORFs under test. In parallel, the β -actin gene of *S. asotus* was used as an internal reference for standardizing the expression of target genes. The primer pairs used for qRT-PCR are given in Table 1.

2.7. Statistical Analysis. The results are presented as means \pm SE. Analysis of statistical differences among the quantity traits was performed by Tukey HSD multiple comparison tests using GraphPad Prism 6 statistical software (GraphPrism Software, La Jolla, California, USA).

3. Results

3.1. Sexual Dimorphic Length Traits Analysis. The 14 length traits were analyzed and are shown in Table 2. In 3 months, there were no differences in body length and body width between male and female *S. asotus*. However,

TABLE 1: The primers information used in this study.

Gene	Primers	Product size (bp)
<i>GH</i>	F: TCAGGTTTCCCTCGGTTAGG R: ATGGGCATCGGTGTGCTTAT	118
<i>GHR</i>	F: GATTTGCGTCCAGAGCTCTAC R: ACGAGATTGCTGAACAGGAG	120
<i>IGF-1</i>	F: TCATCATCTCTGCCCCAG R: CAGGCAGTTGGTAGTGCAGG	116
β -actin	F: AAGATCATTGCCCCACCTGA R: CCTGCTTGCTGATCCACATC	100

the traits related to head shapes, including head depth, head length, head width, postorbital head length, and interorbital width, were markedly different ($P < 0.05$ or $P < 0.01$) between the sexes. In 5 months, except for eye diameter, all traits were notably different between males and females ($P < 0.05$ or $P < 0.01$). The female body length was significantly longer than the male ($P < 0.01$). In 7 months, except for caudal peduncle depth, all traits were significantly different between males and females ($P < 0.05$ or $P < 0.01$). In female *S. asotus*, body length, head width, postorbital head length, eye diameter, interorbital width, caudal peduncle depth, caudal fin length, and pelvic fin precoxal length in 7 months were significantly greater than in 3 and 5 months. In male *S. asotus*, body length, body width, head depth, head width, head length, postorbital head length, eye diameter, interorbital width, caudal peduncle depth, and caudal fin length in 7 months were significantly greater than in 5 and 3 months.

3.2. Sexual Dimorphic Weight Traits Analysis. The 9 weight traits were analyzed and are shown in Table 3. There were no differences in spleen weight between males and females at 3 months, 5 months, or 7 months, although the mean values of females were greater than those of males. In 3 months, all traits showed no significant differences between male and female fish. In 5 and 7 months, weight, visceral weight, eviscerated weight, gonad weight, intestines weight, kidney weight, and liver weight in females were significantly greater than in males ($P < 0.05$ or $P < 0.01$). In females, body weight, eviscerated weight, and kidney weight in 7 months were significantly greater than in 3 or 5 months ($P < 0.05$ or $P < 0.01$). In males, there were no significant differences at 3, 5, or 7 months, although the mean body weight and other traits were greater than at 3 or 5 months.

3.3. Sexual Dimorphic Condition Factors and Maturity. At 7 months, the condition factors of females were significantly greater than those of males, while there were no differences between males and females at 3 and 5 months (Figure 1(a)). The maturities of females were significantly greater than males ($P < 0.01$) at 5 and 7 months, while the female's at 3 months was significantly less than the male ($P < 0.05$) (Figure 1(b)).

TABLE 2: Statistical analysis of length traits between female and male in *S. asotus*.

Variables	3 months		5 months		7 months	
	Female (n = 83)	Male (n = 91)	Female (n = 112)	Male (n = 88)	Female (n = 102)	Male (n = 98)
Body length (mm)	278.51 ± 3.75 ^{de}	262.37 ± 4.53 ^c	327.13 ± 5.78 ^b	286.55 ± 4.99 ^d	352.17 ± 2.99 ^a	307.47 ± 2.36 ^c
Body depth/mm (mm)	41.38 ± 0.65 ^b	35.27 ± 0.86 ^c	47.12 ± 1.71 ^a	37.41 ± 0.95 ^{bc}	47.21 ± 0.67 ^a	39.46 ± 0.54 ^{bc}
Body width (mm)	29.7 ± 0.67 ^b	26.44 ± 0.59 ^{bc}	35.64 ± 1.05 ^a	27.34 ± 0.64 ^c	35.55 ± 0.53 ^a	30.6 ± 0.52 ^b
Head depth (mm)	29.12 ± 0.45 ^b	26.41 ± 0.58 ^c	33.26 ± 0.9 ^a	27.16 ± 0.48 ^{bc}	32.92 ± 0.57 ^a	29.91 ± 0.39 ^b
Head length (mm)	62.54 ± 0.84 ^{bc}	55.53 ± 0.84 ^d	73.87 ± 1.65 ^a	58.55 ± 1.12 ^{cd}	75.70 ± 0.79 ^a	64.11 ± 0.54 ^b
Head width (mm)	40.28 ± 0.65 ^{cd}	36.30 ± 1.13 ^e	46.12 ± 1.14 ^b	36.94 ± 0.61 ^{de}	49.61 ± 0.61 ^a	42.87 ± 0.50 ^{bc}
Snout length (mm)	21.14 ± 0.44 ^{cd}	19.57 ± 0.55 ^d	24.51 ± 0.64 ^{ab}	20.05 ± 0.58 ^{cd}	27.42 ± 0.35 ^a	22.67 ± 0.4 ^{bc}
Postorbital head length (mm)	36.62 ± 0.72 ^c	33.62 ± 0.58 ^d	41.46 ± 1.03 ^b	34.16 ± 0.63 ^d	45.53 ± 0.48 ^a	38.38 ± 0.38 ^c
Eye diameter (mm)	4.91 ± 0.18 ^e	5.34 ± 0.17 ^{de}	6.32 ± 0.16 ^c	5.92 ± 0.17 ^{cd}	7.87 ± 0.12 ^a	7.12 ± 0.09 ^b
Interorbital width (mm)	28.36 ± 0.47 ^c	25.44 ± 0.48 ^d	31.40 ± 0.78 ^b	27.36 ± 0.43 ^{cd}	34.07 ± 0.37 ^a	27.24 ± 0.32 ^{cd}
Caudal peduncle depth (mm)	14.51 ± 0.31 ^{bc}	12.38 ± 0.40 ^d	15.13 ± 0.37 ^b	13.73 ± 0.32 ^{cd}	18.18 ± 0.24 ^a	17.02 ± 0.29 ^a
Caudal fin length (mm)	28.58 ± 0.57 ^c	25.17 ± 0.52 ^d	34.41 ± 0.87 ^b	31.05 ± 0.66 ^c	37.63 ± 0.34 ^a	34.04 ± 0.33 ^b
Pelvic fin precoxal length (mm)	105.26 ± 1.76 ^{cd}	98.11 ± 1.65 ^d	124.50 ± 1.60 ^b	105.86 ± 2.05 ^c	141.32 ± 1.70 ^a	111.93 ± 0.95 ^c
Length between pelvic and anal fin (mm)	9.20 ± 0.30 ^c	9.00 ± 0.34 ^c	10.71 ± 0.41 ^{ab}	9.32 ± 0.42 ^c	11.93 ± 0.15 ^a	10.11 ± 0.18 ^{bc}

Note. The data are expressed by the mean ± standard error; the HSD was used to put up multiple comparisons. Significant differences are indicated by lowercases ($P < 0.05$ or $P < 0.01$).

TABLE 3: The statistical analysis of anatomical characteristics between female and male populations in *S. asotus*.

Variables	3 months		5 months		7 months	
	Female (n = 83)	Male (n = 91)	Female (n = 112)	Male (n = 88)	Female (n = 102)	Male (n = 98)
Weight (g)	199.04 ± 13.05 ^c	137.47 ± 10.37 ^c	364.65 ± 41.42 ^b	166.31 ± 15.81 ^c	530.13 ± 25.22 ^a	234.15 ± 32.22 ^c
Visceral weight (g)	17.5 ± 1.46 ^b	11.01 ± 1.81 ^b	42.02 ± 2.20 ^a	15.51 ± 1.38 ^b	47.13 ± 2.75 ^a	17.82 ± 1.63 ^b
Eviscerated weight (g)	185.07 ± 10.47 ^c	127.68 ± 7.61 ^c	291.81 ± 23.10 ^b	160.93 ± 16.98 ^c	413.76 ± 21.13 ^a	202.06 ± 27.07 ^c
Fat weight (g)	2.47 ± 0.57 ^b	1.93 ± 0.42 ^b	5.32 ± 1.43 ^{ab}	1.79 ± 0.75 ^b	7.47 ± 1.00 ^a	1.67 ± 0.63 ^b
Gonad weight (g)	1.52 ± 0.24 ^b	1.76 ± 0.22 ^b	11.62 ± 1.25 ^a	2.08 ± 0.49 ^b	14.10 ± 1.13 ^a	2.11 ± 0.3 ^b
Intestines weight (g)	5.16 ± 0.34 ^b	4.23 ± 0.58 ^b	10.48 ± 0.76 ^a	4.86 ± 0.46 ^b	10.81 ± 0.61 ^a	4.97 ± 0.31 ^b
Kidney weight (g)	1.34 ± 0.37 ^c	0.81 ± 0.23 ^c	3.33 ± 0.22 ^b	1.38 ± 0.12 ^c	4.70 ± 0.34 ^a	1.83 ± 0.17 ^c
Liver weight (g)	3.69 ± 0.50 ^b	2.85 ± 0.32 ^b	7.81 ± 1.01 ^a	3.34 ± 0.37 ^b	9.81 ± 0.91 ^a	3.60 ± 0.43 ^b
Spleen weight (g)	0.64 ± 0.36	0.34 ± 0.17	0.61 ± 0.11	0.38 ± 0.02	0.66 ± 0.05	0.35 ± 0.07

Note. The data were expressed by the mean ± standard error; the HSD was used to put up multiple comparisons. Significant differences are indicated by lowercases ($P < 0.05$ or $P < 0.01$).

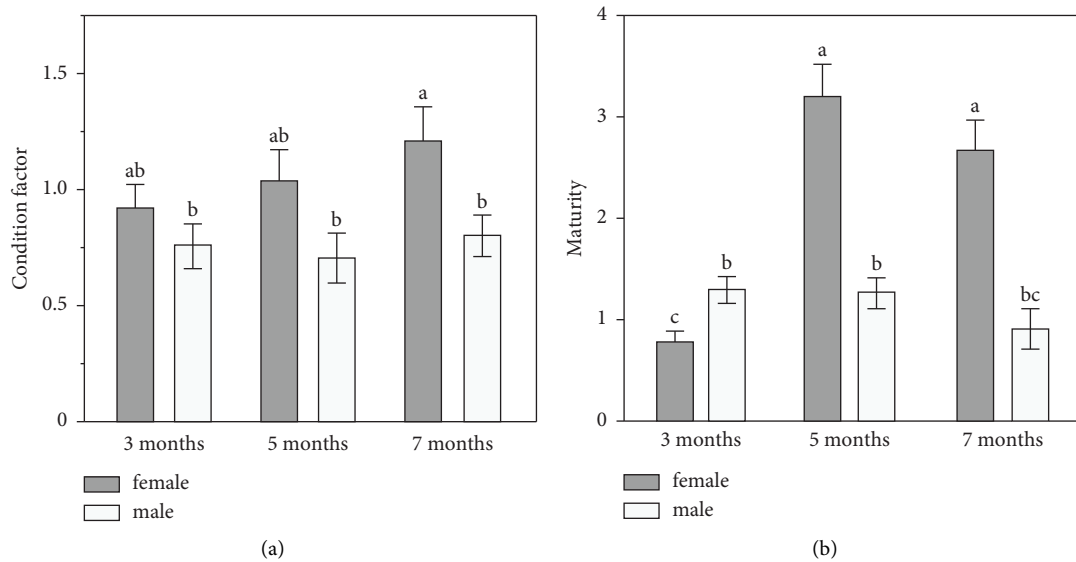


FIGURE 1: Sexual dimorphic condition factor and maturity in 3, 5, and 7 months. (a) Condition factor of male and female *S. asotus* during 3, 5, and 7 months. (b) Maturity of male and female during 3, 5, and 7 months. Significant differences are indicated by lowercases above the error bars ($P < 0.05$ or $P < 0.01$).

3.4. Sexual Dimorphic Allometric Scaling Relative to Body Weight. Joint static allometric analyses were performed on 14 morphological indicators relative to the body weight of female and male *S. asotus* at 3, 5, and 7 months (Table 4). In the three stages, the allometric scaling value of body length was $b_1 > 1$, indicating that the body length of females grew relatively faster than body weight from 3 months to 7 months, while the body length of males grew more slowly than body weight ($b_1 < 1$). In 3 months, female *S. asotus* showed significant allometric relationships for body length, body width, head length, head depth, and pelvic fin precoxal length relative to body weight. Male *S. asotus* showed slow allometric growth rates in body length, body depth, head depth, snout length, interorbital width, and caudal fin length compared to body weight. In 5 months, male *S. asotus* showed a greater allometric growth rate in head length than body weight. The snout length, caudal peduncle depth, caudal fin length, and pelvic fin precoxal length in females and the body width, snout length, and pelvic fin precoxal length in males showed slow allometric relationships related

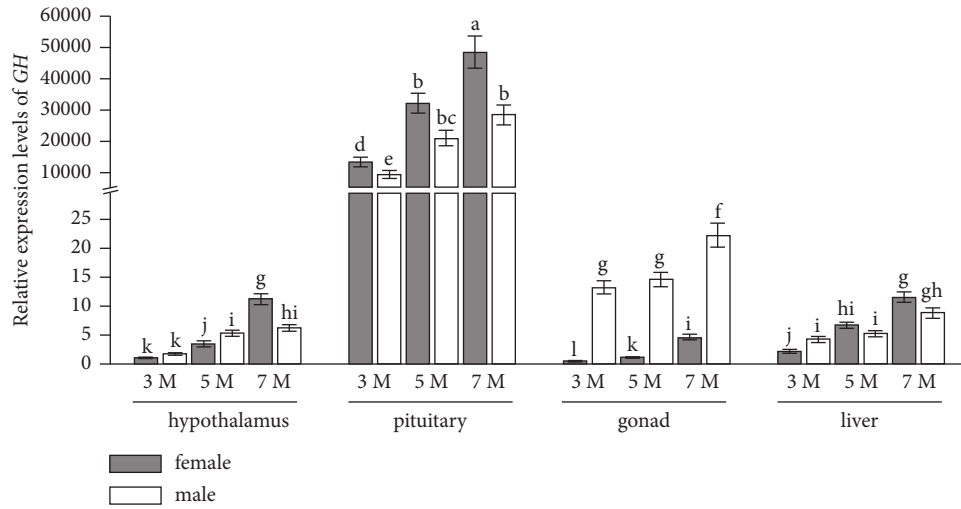
to body weight. In 7 months, male body length had a constant growth relationship with body weight. The female allometric growth rates of body depth, head depth, eye diameter, and pelvic fin precoxal length were slower than body weight. The allometric growth rates of head length, head depth, head width, eye diameter, caudal fin length, and pelvic fin precoxal length in males were slower than body weight.

3.5. Sexual Dimorphic Expression of GH/IGF Axis Genes. The relative expression of *GH-1*, *IGF-1*, and *GHR* in the hypothalamus, pituitary, gonad, and liver of male and female *S. asotus* at 3, 5, and 7 months was detected and is shown in Figure 2. The results showed that the expressions of *GH-1*, *IGF-1*, and *GHR* were various in different tissues and represented gender-specific expression patterns. For the *GH* gene, the expression levels in the pituitary were significantly greater than in the hypothalamus, liver, and gonad, with a high expression pattern of female preference. For *IGF-1*,

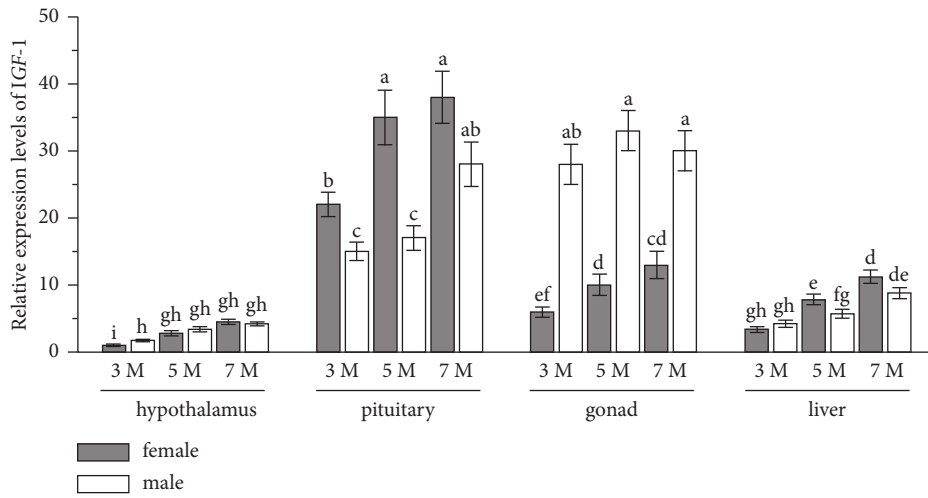
TABLE 4: Allometric analysis of morphological traits to weight between male and female *S. asotus* in 3, 5, and 7 months.

Independent variable	Allometry scaling	3 months				5 months				7 months			
		Female		Male		Female		Male		Female		Male	
		Est.	Std. E	Est.	Std. E	Est.	Std. E	Est.	Std. E	Est.	Std. E	Est.	Std. E
Body length	b_1	1.471	0.117	0.622	0.110	1.780	0.857	0.707	0.248	1.807	0.218	1.044	0.205
Body width	b_2	0.504	0.097					0.690	0.300				
Body depth	b_3			0.917	0.331					0.661	0.059	0.810	0.065
Head length	b_4	0.864	0.208					1.425	0.604			0.770	0.057
Head width	b_5											0.583	0.041
Head depth	b_6	0.719	0.188	0.207	0.048					0.189	0.083		
Snout length	b_7			0.517	0.224	-0.360	0.054	-0.881	0.341				
Postorbital head length	b_8												
Eye diameter	b_9									0.464	0.063	0.278	0.044
Interorbital width	b_{10}			0.530	0.249								
Caudal peduncle depth	b_{11}					0.257	0.089						
Caudal fin length	b_{12}			0.473	0.060	0.153	0.056	0.408	0.116	0.614	0.078	0.537	0.062
Pelvic fin precoxal length	b_{13}					0.237	0.094						
Length between pelvic fin and anal fin	b_{14}	0.162	0.057										

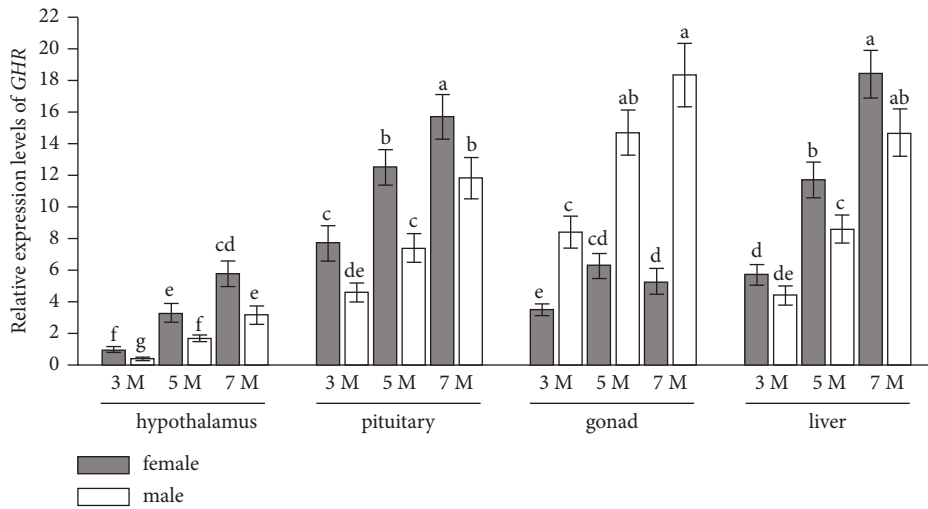
Note. Est. means estimate and Std. E means standard error.



(a)



(b)



(c)

FIGURE 2: Sexual dimorphic expression of GH/IGF axis genes in 3, 5, and 7 months. (a)–(c) Expressions of GH, IGF-1, and GHR genes in the hypothalamus, pituitary, gonad, and liver during 3, 5, and 7 months of male and female *S. asotus*. β -Actin gene was used as the internal control. Significant differences are indicated by lowercases above the error bars ($P < 0.05$ or $P < 0.01$).

the expression levels in the pituitary and testis were significantly higher than those in the hypothalamus and liver. For *GHR*, the expression levels in the pituitary, testis, and liver were significantly higher than the hypothalamus at 5 and 7 months. The three genes showed higher expression levels in the hypothalamus, pituitary, and liver of females and male-biased high expression patterns in the testis.

4. Discussion

The catfish order is one large family of teleost, containing about 3,407 species currently recognized [45]. Sexual dimorphisms in growth traits are expected in the catfish family. Lu et al. [46] found that in *P. fulvidraco*, the males grow faster than the females. However, our study found that the female *S. asotus* grows significantly faster and is larger than the male at 7 months. Consistently, Shen et al. [1] found that the mature females of *S. asotus* are larger than the males, with a more than 2-fold body weight. These results indicate that there is a female-biased sexual dimorphism in *S. asotus*. We also found a variety of allometric growth patterns of sexual dimorphism in the growing process of juvenile *S. asotus*. At 3 months, there were no significant differences in body weight and length. Meanwhile, the sexual characteristics are not obvious. It is challenging to identify males or females by phenotypic characteristics or specific sexual characteristics, while we also found that several local quantifiable characters focusing on the head were significantly greater in females compared to males in juvenile *S. asotus*. The *D. labrax* exhibits female-biased sexual size dimorphism (SSD) early in development (from 103 dph to 165 dph) [21]. Our study also confirmed that the female-biased SSD pattern in *S. asotus* is strongly influenced by early growth differences between sexes. Females had a larger head shape than males, likely due to adaptations to female mouthbrooder foraging strategies [47]. SSD has captivated considerable curiosity for farmed fish production [48]. The head character differences between male and female *S. asotus* in 3 months could provide potential morphological markers for filtering the female group to improve production efficiency for aquaculture.

Fish generally show allometric relationships during the different stages of development [49]. Allometric relationships between body properties are helpful for a lot of studies, such as estimation of biomass, growth, population structure, and bioenergetic modeling studies [50]. The various brain size-body length allometries could reflect the climate changes [51]. However, there need to be more knowledge on the allometric analysis between sexes at different early development stages, which could help recognize the potential different growth patterns between males and females in *S. asotus*. The length-weight relationship (LWR) is the key to providing useful information on growth patterns in fish [52]. In this study, despite the slight difference in body length and weight between male and female *S. asotus* in 3 months, there was a positive LWR found in females ($b_1 > 1$) and a negative LWR in males ($b_1 < 1$), implying sexually dimorphic

growth patterns in the early development stage of the fish. It also implies that the growth in the size of the fish is not proportionate to the increase in weight [53]. The more rapid increase in body length of females compared with body weight at the early development stage could determine the large body size in mature females. Meanwhile, the weight gains in females were significantly greater than in males with greater condition factors (K) at 5 and 7 months, which indicated the rapid weight gains in juvenile females. A larger body size with a larger mouth shape could help females get more food and be more competitive compared to males. Thus, the lifetime reproductive success of males is limited by access to females. In contrast, females are not limited by their access to males, which leads to an unbalance in the number of individuals of each sex in the population [54].

Most morphological and physiological differences between females and males are caused by the differential expression of genes in both sexes [55, 56]. Fish ingestion and digestion, growth-related gene expressions, sex hormone levels, and energy allocation for growth and reproduction could all lead to sexual dimorphism in growth traits [33]. Generally, the growth hormone and insulin-like growth factor axis (GH/IGF axis) are the central hormone systems that regulate the growth of individual vertebrates. In *P. fulvidraco*, *GH* and *IGF-1/2* gene expressions in male juveniles were significantly higher than those in females, indicating that the differential expression of crucial hormone genes on the GH/IGF axis could induce a faster growth rate in males [34]. However, our results showed that the expressions of *GH* and *IGF-1* were significantly higher in the hypothalamus, pituitary, and liver. These results are consistent with the expression patterns in *C. semilaepis* [57], indicating the universally high expression patterns in females where the females are larger than males. As an essential digestive and metabolic organ, the liver plays a crucial role in regulating fish growth. We found the *GHR* gene was significantly higher in the liver, indicating increased activity of the growth hormone (GH) [58]. Meanwhile, we found that the three genes were highly expressed in the testis, indicating that the expression levels of the three genes are essential in the regulation of gonad maturation [59]. In addition, in the hypothalamus-pituitary-gonad (HPG) system, circulating pituitary GH stimulates the production of IGF-1, which is mainly responsible for growth; IGF-2, which is less studied in teleosts [60], should be detected in future studies for its role in the early differentiation of sex.

5. Conclusion

The dimorphic growth between females and males observed in *S. asotus* from 3 months to 7 months demonstrates that length traits focusing on the head show significant sex differences in early development. The growth rate of juvenile female *S. asotus* is higher than that of male individuals, which could be interpreted by reproductive selection. The key endocrine genes of the GH/

IGF axis could be involved in the sexual size dimorphism of *S. asotus*. However, the molecular mechanism of growth differential regulation between male and female *S. asotus* needs further study.

Data Availability

The gene expression data and the phenotypic data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Zhigang Qiao and Sijia Liu conceived, designed, and supervised the study. Muzi Li, Miao Yu, Meng Zhang, Lei Wang, and Hongxia Jiang conducted the biological experiment and analyzed the data. All the authors wrote the manuscript and were involved in the interpretation of the preliminary data. They have read and approved the manuscript, are aware of the submission for publication, and agree to be listed as co-authors.

Acknowledgments

The authors are grateful for the technical assistance and material support received from the staffs of the Qixian County Qihe Crucian Carp Breeding Co., Ltd. The authors give special thanks to J. Gao. This research was supported by the Open Project of the State Key Laboratory of Freshwater Ecology and Biotechnology (2017FB08), the Key Scientific Research Project of Henan Universities (17B240003), the Open Fund Project of Tianjin Key Laboratory of Aquatic Ecology and Aquaculture (TJAE201806), and the Qinghai Kunlun Talent Plan Project.

References

- [1] F. Shen, Y. Long, F. Li et al., "De novo transcriptome assembly and sex-biased gene expression in the gonads of Amur catfish (*Silurus asotus*)," *Genomics*, vol. 112, no. 3, pp. 2603–2614, 2020.
- [2] Z. G. Qiao, J. P. Zhang, J. Y. Niu, and W. Wang, "Effects of starvation and refeeding on the blood indices of *Silurus asotus*," *Acta Hydrobiologica Sinica*, vol. 32, no. 5, pp. 631–636, 2008.
- [3] G. Chen, X. J. Li, Z. G. Qiao, and X. L. Peng, "Induction of gynogenesis of *Silurus asotus* using cold shock," *Agricultural Science & Technology*, vol. 9, no. 2, pp. 122–125, 2008.
- [4] E. Saillant, A. Fostier, B. Menu, P. Haffray, and B. Chatain, "Sexual growth dimorphism in sea bass *Dicentrarchus labrax*," *Aquaculture*, vol. 202, no. 3–4, pp. 371–387, 2001.
- [5] J. Mei and J. Gui, "Genetic basis and biotechnological manipulation of sexual dimorphism and sex determination in fish," *Science China Life Sciences*, vol. 58, pp. 124–136, 2015.
- [6] A. Hidir, M. A. Aaqillah-Amr, M. N. Azra et al., "Sexual dimorphism of mud crab, genus *Scylla* between sexes based on morphological and physiological characteristics," *Aquaculture Research*, vol. 52, no. 12, pp. 5943–5961, 2021.
- [7] R. Shine, "Sexual selection and sexual dimorphism in the Amphibia," *Copeia*, vol. 2, pp. 297–306, 1979.
- [8] C. A. Goudie, *Production of Monosex Populations: The Channel Catfish Model*, pp. 150–155, International Fish Physiology Symposium, Vancouver, Canada, 1994.
- [9] B. Zhu and C. Xie, "A study of feeding, reproduction, age and growth of Dark sleeper *Odontobutis obscura* in Bao'An lake," *Acta Hydrobiologica Sinica*, vol. 23, pp. 316–323, 1999.
- [10] J. A. Beardmore, G. C. Mair, and R. I. Lewis, "Monosex male production in finfish as exemplified by tilapia: applications, problems, and prospects," *Reproductive Biotechnology in Finfish Aquaculture*, vol. 197, no. 1–4, pp. 283–301, 2001.
- [11] Z. H. Lin and H. Z. Lei, "Sexual dimorphism and female reproductive characteristics of *Pseudobagrus fulvidraco*," *Chinese Journal of Zoology*, vol. 39, no. 6, pp. 13–17, 2004.
- [12] D. Wang, H. L. Mao, H. X. Chen, H. Q. Liu, and J. F. Gui, "Isolation of Y-and X-linked SCAR markers in yellow catfish and application in the production of all-male populations," *Animal Genetics*, vol. 40, no. 6, pp. 978–981, 2009.
- [13] Z. H. Lin, H. Z. Lei, L. L. Chen, X. L. Fan, Z. H. Bao, and B. R. Gao, "Sexual dimorphism in morphological traits and female individual fecundity of *Abbottina rivularis*," *Sichuan Journal of Zoology*, vol. 26, no. 4, pp. 910–913, 2007.
- [14] R. Shine, "Ecological causes for the evolution of sexual dimorphism: a review of the evidence," *The Quarterly Review of Biology*, vol. 64, no. 4, pp. 419–461, 1989.
- [15] V. J. Bye and R. F. Lincoln, "Commercial methods for the control of sexual maturation in rainbow trout (*salmo gairdneri* r.)," *Aquaculture*, vol. 57, no. 1–4, pp. 299–309, 1986.
- [16] D. Q. Xu, Z. H. Lin, and H. Z. Lei, "Sexual dimorphism in morphological traits and female individual fecundity of *Acrossocheilus wenchowensis*," *Journal of Shanghai Jaotong University (Agricultural science)*, vol. 24, no. 4, pp. 335–340, 2006.
- [17] M. Yoneda, Y. Kurita, D. Kitagawa et al., "Age validation and growth variability of Japanese flounder *Paralichthys olivaceus* off the Pacific coast of northern Japan," *Fisheries Science (Carlton, Australia)*, vol. 73, no. 3, pp. 585–592, 2007.
- [18] S. L. Chen, J. Li, S. P. Deng et al., "Isolation of female-specific AFLP markers and molecular identification of genetic sex in half-smooth tongue sole (*Cynoglossus semilaevis*)," *Marine Biotechnology*, vol. 9, no. 2, pp. 273–280, 2007.
- [19] X. L. Fan, Z. H. Lin, X. G. Hu, H. Z. Lei, and X. Li, "Sexual dimorphism and female reproductive outputs of the ovoviparous and invasive mosquitofish *Gambusia affinis*," *Acta Ecologica Sinica*, vol. 36, no. 9, pp. 2497–2504, 2016.
- [20] R. Shine, "The evolution of large body size in females: a critique of Darwin's "Fecundity Advantage" model," *The American Naturalist*, vol. 131, no. 1, pp. 124–131, 1988.
- [21] S. Faggion, M. Vandeputte, A. Vergnet et al., "Sex dimorphism in European sea bass (*Dicentrarchus labrax* L.): new insights into sex-related growth patterns during very early life stages," *PLoS One*, vol. 16, no. 4, Article ID 239791, 2021.
- [22] R. Bonduriansky, "Sexual selection and allometry: a reappraisal of the evidence and ideas," *Evolution*, vol. 61, no. 4, pp. 838–849, 2007.
- [23] J. S. Huxley and G. Teissier, "Terminology of relative growth," *Nature*, vol. 137, no. 3471, pp. 780–781, 1936.
- [24] Y. X. Liu, Y. Liu, Y. J. Liu, and R. Q. Yang, "Allometric analysis of body weight and morphological traits for Japanese flounder (*Paralichthys olivaceus*)," *South China Fisheries Science*, vol. 12, no. 1, pp. 36–42, 2016.

- [25] J. Zhao, S. Li, L. Wang, L. Jiang, R. Yang, and Y. Cui, "Genome-wide random regression analysis for parent-of-origin effects of body composition allometries in mouse," *Scientific Reports*, vol. 7, no. 1, pp. 1–9, 2017.
- [26] H. J. Gao, Y. X. Liu, T. T. Zhang, R. Q. Yang, and H. M. Yang, "Statistical models for jointly analyzing multiple allometries," *Journal of Theoretical Biology*, vol. 318, pp. 205–209, 2013.
- [27] J. Zhao, Y. Zhao, Z. Song, H. Liu, Y. Liu, and R. Yang, "Genetic analysis of the main growth traits using random regression models in Japanese flounder (*Paralichthys olivaceus*)," *Aquaculture Research*, vol. 49, no. 4, pp. 1504–1511, 2018.
- [28] J. He, H. Gao, P. Xu, and R. Yang, "Genetic parameters for different growth scales in GIFT strain of Nile tilapia (*Oreochromis niloticus*)," *Journal of Animal Breeding and Genetics*, vol. 132, no. 6, pp. 467–474, 2015.
- [29] T. M. Williams and S. B. Carroll, "Genetic and molecular insights into the development and evolution of sexual dimorphism," *Nature Reviews Genetics*, vol. 10, pp. 797–804, 2009.
- [30] L. Zhou, R. Yang, H. Tian et al., "Sexual dimorphism in *Odontobutis sinensis* brain-pituitary-gonad axis and liver highlighted by histological and transcriptomic approach," *Gene*, vol. 819, Article ID 146264, 2022.
- [31] J. J. Wu, Y. L. Zhou, Z. W. Wang et al., "Comparative transcriptome analysis reveals differentially expressed genes and signaling pathways between male and female red-tail catfish (*Mystus wyckioides*)," *Marine Biotechnology*, vol. 21, no. 4, pp. 463–474, 2019.
- [32] Z. Liu, S. Mohan, and S. Yakar, "Does the GH/IGF-1 axis contribute to skeletal sexual dimorphism? Evidence from mouse studies," *Growth Hormone & IGF Research*, vol. 27, pp. 7–17, 2016.
- [33] X. Dai, W. Zhang, Z. Zhuo, J. He, and Z. Yin, "Neuroendocrine regulation of somatic growth in fishes," *Science China Life Sciences*, vol. 58, no. 2, pp. 137–147, 2015.
- [34] M. Wenge, W. Junjie, Z. Jin, H. Yan, G. Jianfang, and M. Jie, "Sex differences in the expression of GH/IGF axis genes underlie sexual size dimorphism in the yellow catfish (*Pelteobagrus fulvidraco*)," *Science China Life Sciences*, vol. 59, no. 4, pp. 431–433, 2016.
- [35] M. Reinecke, B. T. Björnsson, W. W. Dickhoff et al., "Growth hormone and insulin-like growth factors in fish: where we are and where to go," *General and Comparative Endocrinology*, vol. 142, no. 1–2, pp. 20–24, 2005.
- [36] J. E. Puche and I. Castilla-Cortázar, "Human conditions of insulin-like growth factor-I (IGF-I) deficiency," *Journal of Translational Medicine*, vol. 10, no. 1, pp. 1–29, 2012.
- [37] M. Nipkow, E. Wirthgen, P. Luft, A. Rebl, A. Hoeflich, and T. Goldammer, "Characterization of igf1 and igf2 genes during maraena whitefish (*Coregonus maraena*) ontogeny and the effect of temperature on embryogenesis and igf expression," *Growth Hormone & IGF Research*, vol. 40, pp. 32–43, 2018.
- [38] J. H. Fowke, C. E. Matthews, H. Yu et al., "Racial differences in the association between body mass index (BMI) and serum IGF-1, IGF-2, and IGFBP-3," *Endocrine-Related Cancer*, vol. 17, no. 1, p. 51, 2010.
- [39] C. L. Brown, E. M. V. Cruz, R. B. Bolivar, and R. J. Borski, "Production, growth, and insulin-like growth factor-I (IGF-I) gene expression as an instantaneous growth indicator in Nile tilapia *Oreochromis niloticus*," *Functional Genomics in Aquaculture*, vol. 79, 2012.
- [40] M. Biellohuby, S. H. Zarkesh-Esfahani, J. Manolopoulou et al., "Validation of serum IGF-I as a biomarker to monitor the bioactivity of exogenous growth hormone agonists and antagonists in rabbits," *Disease models & mechanisms*, vol. 7, no. 11, pp. 1263–1273, 2014.
- [41] Y. Z. Mao, H. S. Wen, H. R. Lin, and C. Y. Li, "Induction of ovulation and spawn by LHRH-A and DOM in catfish, *Silurus asotus*," *Journal of Fishery Sciences of China*, vol. 8, no. 2, pp. 48–51, 2001.
- [42] Q. Wang, Y. P. Li, J. G. Wang, and K. H. Min, "Sexual dimorphism in morphological traits and female individual fecundity of *Rhodeus sinensis*," *Jiangsu Agricultural Sciences*, vol. 41, no. 2, pp. 200–203, 2013.
- [43] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method," *A Companion to Methods in Enzymology*, vol. 25, no. 4, 2001.
- [44] W. Rychlik, "OLIGO 7 primer analysis software," *PCR Primer Design*, pp. 35–59, 2007.
- [45] J. W. Armbruster, "Global catfish biodiversity," *American Fisheries Society Symposium*, vol. 77, pp. 15–37, 2011.
- [46] J. Lu, M. Zheng, J. Zheng et al., "Transcriptomic analyses reveal novel genes with sexually dimorphic expression in yellow catfish (*Pelteobagrus fulvidraco*) brain," *Marine Biotechnology*, vol. 17, no. 5, pp. 613–623, 2015.
- [47] J. Herler, M. Kerschbaumer, P. Mitteroecker, L. Postl, and C. Sturmbauer, "Sexual dimorphism and population divergence in the Lake Tanganyika cichlid fish genus *Tropheus*," *Frontiers in Zoology*, vol. 7, no. 1, pp. 1–10, 2010.
- [48] H. Chen, D. Jiang, Z. Li et al., "Comparative physiological and transcriptomic profiling offers insight into the sexual dimorphism of hepatic metabolism in size-dimorphic spotted scat (*Scatophagus argus*)," *The Life*, vol. 11, no. 6, p. 589, 2021.
- [49] R. H. Devlin, W. E. Vandersteen, M. Uh, and E. D. Stevens, "Genetically modified growth affects allometry of eye and brain in salmonids," *Canadian Journal of Zoology*, vol. 90, pp. 193–202, 2012.
- [50] F. L. Schaafsma, C. L. David, D. Kohlbach et al., "Allometric relationships of ecologically important Antarctic and Arctic zooplankton and fish species," *Polar Biology*, vol. 45, no. 2, pp. 203–224, 2022.
- [51] M. Liu, J. Jia, H. Wang, and L. Wang, "Allometric model of brain morphology of *Hemiculter leucisculus* and its variation along climatic gradients," *Journal of Anatomy*, vol. 241, no. 2, pp. 259–271, 2022.
- [52] K. A. Ighwela, A. B. Ahmed, and A. B. Abol-Munafi, "Condition factor as an indicator of growth and feeding intensity of Nile Tilapia fingerlings (*Oreochromis niloticus*) feed on different levels of maltose American-urasian," *American-Eurasian Journal of Agricultural & Environmental Sciences*, vol. 11, no. 4, pp. 559–563, 2011.
- [53] R. Riedel, L. M. Caskey, and S. H. Hurlbert, "Length-weight relations and growth rates of dominant fishes of the Salton Sea: implications for predation by fish-eating birds," *Lake and Reservoir Management*, vol. 23, pp. 528–535, 2007.
- [54] E. Forsgren, J. D. Reynolds, A. Berglund, and R. D. Mooi, "Behavioural Ecology of reproduction in fish," *Handbook of Fish Biology and Fisheries*, vol. 1, pp. 225–247, 2008.
- [55] H. Ellegren and J. Parsch, "The evolution of sex-biased genes and sex-biased gene expression," *Nature Reviews Genetics*, vol. 8, no. 9, pp. 689–698, 2007.
- [56] S. Grath and J. Parsch, "Sex-biased gene expression," *Annual Review of Genetics*, vol. 50, pp. 29–44, 2016.

- [57] Q. Ma, S. F. Liu, Z. M. Zhuang et al., "Molecular cloning, expression analysis of insulin-like growth factor I (IGF-I) gene and IGF-I serum concentration in female and male Tongue sole (*Cynoglossus semilaevis*)," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 160, no. 4, pp. 208–214, 2011.
- [58] S. El-Kassas, S. E. Abdo, W. Abosheashaa et al., "Growth performance, serum lipid profile, intestinal morphometry, and growth and lipid indicator gene expression analysis of mono-sex Nile tilapia fed *Moringa oleifera* leaf powder," *Aquaculture Reports*, vol. 18, Article ID 100422, 2020.
- [59] P. S. Prado, A. P. B. Pinheiro, A. A. Weber, N. Bazzoli, and E. Rizzo, "Expression patterns and immunolocalisation of IGF-I and IGF-II in male and female gonads of the Neotropical characid fish *Astyanax fasciatus*," *Fish Physiology and Biochemistry*, vol. 45, no. 1, pp. 167–176, 2019.
- [60] E. T. Won, J. D. Douros, D. A. Hurt, and R. J. Borski, "Leptin stimulates hepatic growth hormone receptor and insulin-like growth factor gene expression in a teleost fish, the hybrid striped bass," *General and Comparative Endocrinology*, vol. 229, pp. 84–91, 2016.