Hindawi
Aquaculture Research
Volume 2023, Article ID 4767850, 10 pages
https://doi.org/10.1155/2023/4767850

Research Article

Early Development of the Spinal Vertebrae and Appendicular Skeleton in *Miichthys miiuy* Larvae

Fengfang Zhou,[1,2,3] **Weiqing Huang**,[1,2,4] **Xiujuan Shan**,[5] **Lei Shi**,[1] **and Zheng Liu**[1]

[1]*School of Life Sciences, Ningde Normal University, Ningde 352100, China*
[2]*Engineering Research Center of Mindong Aquatic Product Deep-Processing, Fujian Province University, Ningde 352100, China*
[3]*State Oceanic Administration Hercynian Special Biological Germplasm Resources and Biological Product Development Public Service Platform, Ningde 352100, Fujian, China*
[4]*Ningde Dingcheng Fishery Company Limited, Ningde 352100, China*
[5]*Key Laboratory of Sustainable Development of Marine Fisheries, Ministry of Agriculture and Rural Affairs, Shandong Provincial Key Laboratory of Fishery Resources and Ecological Environment, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China*

Correspondence should be addressed to Weiqing Huang; 393634584@qq.com and Xiujuan Shan; shanxj@ysfri.ac.cn

Received 23 September 2022; Revised 3 January 2023; Accepted 9 January 2023; Published 3 February 2023

Academic Editor: Hamed Ghafarifarsani

We investigate the early developmental characteristics of the skeletal system in *Miichthys miiuy* larvae using a double staining method on the whole larval skeletons (aged 1–30 days). We systematically observed and described the morphological development of both the spinal vertebrae and appendicular skeleton. The results showed that there were 25 spinal vertebrae in *M. miiuy*, which consist of 12 abdominal vertebrae and 13 coccyges; The development of spinal vertebrae began at 12 days after hatching (dah), with the emergence of ossified neural and haemal arches, where ossification appeared at 17 dah; and the appendicular skeletons were developed from the pectoral fins to ventral fins. The coracoid-scapula cartilage was clearly observable at three dah, and fin rays were clearly differentiated at 21 dah. The cartilage of appendicular of the ventral fins was formed until 19 dah, and the ventral fins were completely ossified at 21 dah. Subsequently, the caudal, dorsal, and anal fins appeared at 10, 13, and 14 dah, respectively. Additionally, the appendicular skeleton associated with the movement of *M.miiuy* was completely developed by 28 dah. Finally we observed that there are four types of spinal vertebrae abnormalities during the early development of *M.miiuy*, which included spinal bulging, splitting of vertebrae, and supernumerary and bifurcated haemal and neural spines. Skeletal length and age (in days) were highly correlated ($r^2 = 0.942$). There was a strong relationship between total length and skeletal length ($r^2 = 0.979$) and between body length and skeletal length ($r^2 = 0.9779$).

1. Introduction

In fish, the skeleton plays a pivotal role in interior protection, coordinating movement, respiration, feeding, systematic evolution and classification [1]. Skeletal development is affected by genetic, environmental, and nutritional factors [2]. Further, skeletal deformities seriously affect fish morphology, slowing the growth and lowering the market value of the stock [3]. Many studies have been conducted on the developmental characteristics of fish skeletons. For example, the growth and development of the spinal joints in *Larimichthys crocea* was analysed using the double staining method [4]. In another study, cartilage bone clearing and staining technique were used to describe the skeletal developmental characteristics and skeletal malformations of cobia larvae in detail [5]. At present, fish osteology mainly includes the identification and occurrence of bone deformities, and the effects of genetic, environmental, and nutritional factors on fish deformity and mortality.

Miichthys miiuy (Perciformes, Sciaenidae, Miichthys), is a commercially important offshore warm-water demersal species [6]. The species is mainly distributed in the Zhejiang, Taiwan, Fujian, Shandong, and Jiangsu provinces in China, with the Zhoushan Islands in the Zhejiang province being

the largest fish-producing area [7]. With high nutritional and economic value and rapid growth, *M. miiuy* has become an important stock for capture fisheries and aquaculture. Experimental studies on artificial reproduction and breeding of *M. miiuy* have been conducted in coastal areas such as Fujian and Zhejiang provinces since the 1990s [8]. At present, research on *M.miiuy* is mainly focused on improving aquaculture techniques [9], muscle nutritional composition [10], gene function and regulation [11] and processing technology [12, 13].

The skeletal development of *M.miiuy* has been studied in terms of basic morphology and morphometrics. Here, we study the growth and development patterns in further detail, as well as ecological significance of these patterns, by observing and quantifying the early ontogenesis of both larval and juvenile *M. miiuy* (aged 1–53 days) [14]. Morphological characteristics of the early skeleton development (including hyoid arch, branchial arch, and opercular apparatus) in relation to their feeding habit were observed in *M. miiuy* larvae using standard clearing and staining methods [15]. However, the early development of the spinal vertebrae and appendicular skeleton in *M. miiuy* larvae has not yet been explored.

With the continuous development and expansion of the *M. miiuy* aquaculture industry, the exploration of skeletal developmental are essential for the evidence-based guidance in large-scale aquaculture, particularly for improving breeding and increasing production. The early developmental characteristics of spinal vertebrae and appendicular skeleton in the larval and juvenile *M. miiuy*, as well as the detailed observations and descriptions of some abnormal phenomena during the development process of the vertebrae, were quantified in this study. We aim to provide further understanding of the developmental biology of the species, which will have useful applications for the commercial breeding of high quality *M. miiuy* stock.

2. Materials and Methods

2.1. Ethics Statement. All procedures complied with ethic committee of Ningde Normal University regarding animal experimentation.

2.2. Sample Collection. Larvae of *M. miiuy* in this study were provided by Ning De Ding Cheng Aquatic Product Company Ltd. (Ningde, China). The *M. miiuy* sampling and experiments were conducted in accordance with the ethical requirements and followed the guidelines for the Animal Ethics Committee of Ningde Normal University. Larvae were reared in 40 m^3 tanks with water up to a depth of 1 m. Rearing density was *c.* 125 larvae l^{-1}. During the experimental period, oxygen, salinity, and pH were 7.0–8.5 mg l^{-1}, 22–25, and 6.5–7.0, respectively, and were monitored daily. Larvae were fed rotifers (*Brachionus plicatilis*). Next, 10 larvae aged 1–30 days were randomly sampled at each day posthatching. They were placed in 10% neutral buffered formalin for at least 24 h to ensure complete fixation of samples.

2.3. Larval and Juvenile Skeleton Specimens. The cartilage bone clearing and staining technique was used as described by Huang et al. [4], after some modifications. The specific steps used are as follows:

2.3.1. Fixation and Cleaning. The samples were continually soaked in 10% formalin for three days and then in distilled water for one day.

2.3.2. Graded Dehydration. Samples were dehydrated with graded ethanol (20% ⟶ 40% ⟶ 60% ⟶ 75%) for 12–24 h of each gradient. The dehydration time depended on the sample size. Subsequently, the samples were stored at 4°C and stained in 95% alcohol for another 24 h before soaking.

2.3.3. Cartilage Staining. The cartilage staining solution was prepared by adding 20 ml of glacial acetic acid, 80 ml of 95% alcohol, and 10 mg of Alisin Blue Stain. The samples were stained in this solution for approximately 5 h until the body surface and fin base of the fish was clearly blue.

2.3.4. Neutralization. The samples were soaked in saturated sodium borate solution for 1 day.

2.3.5. Decolouration. The samples were soaked in the mixture of 20 ml of 3% H_2O_2 solution and 80 ml of 1% KOH solution for one day to decolorize.

2.3.6. Bone Staining. The bone staining solution was prepared by adding 5 mg of alizarin red and 100 ml of 1% KOH. The samples were stained in this solution for 5–9 h, depending on the sample volume.

2.3.7. Sample Transparency. The sample was placed in 1% KOH solution for transparency until the surface was unpigmented and the bones could be clearly observed.

2.3.8. Storage. The samples were transferred sequentially into a mixture of glycerol and 1% KOH in a ratio of 1 : 3, 1 : 1, and 3 : 1 to elute excess stain for one day each. The samples were stored in pure glycerine when the fish became transparent.

2.4. Larval and Juvenile Observation of Transparent Specimens. The developmental process and morphological characteristics of the spinal vertebrae and appendicular skeleton were observed using a Leica S9i Digital Stereo Microscope (Leica S9i, Germany), photographed, and recorded. We used the naming conventions for each skeleton as in Deng et al. [16]; and Mao [5]. We named the different vertebrae deformation types according to Gai et al. [13].

2.5. Data Processing. The total length, body length, and skeletal length of larvae and juveniles were measured (the total length: the length from snout to the end of caudal fin; the body length: the length from snout to the front of caudal fin; the skeletal length: the length from the the first abdominal vertebra to the coccyx). Correlations were calculated using the Microsoft Excel software (2010), and recorded photographs were processed using Adobe Photoshop CC (2017). Based on the observation and analysis of those photos, the developmental characteristics of spinal vertebrae and appendicular skeleton, as well as spinal vertebrae abnormalities, were summarized.

3. Results

3.1. Developmental Characteristics of Spinal Vertebrae. The spinal vertebrae of larval *M. miiuy* were clearly visible and the ossification appeared at 17 days, but the development of the spinal vertebrae was completed at 28 days after hatching (dah). The developmental characteristics and spinal joints of spinal vertebrae were observed and counted from 17 to 30 dah. There were 25 spinal vertebrae in *M. miiuy*, which consist of 12 abdominal vertebrae and 13 coccyges.

Ossification did not appear in the skeleton of larval *M. miiuy* at one dah, with a straight notochord and no appendicular skeleton. An uneven notochord was observed at five dah, and the neural arch appeared ahead of the notochord (Figure 1(a)). At 12 dah, the neural arch cartilage on the dorsal spine and haemal arch cartilage on the ventral spine were clearly visible, and a pair of those cartilage arches formed a neural arch, which was located on both sides of the dorsal spine. The caudal part of neural arch was fused, and the vertebral cartilage also became abjoint at this time. With the development of the skeleton, the neural arch and haemal arch were extended to neural and haemal spines, and the vertebral column completely ossified from anterior to posterior (Figure 1(b)). At 17 dah, all joints in spinal vertebrae of larval *M. miiuy* were stained with alizarin red, which indicates that ossification was complete. Additionally, the biconcave joint combined to form the vertebrae and protect the notochord. We clearly observed 25 nodes, neural and haemal spines (Figure 1(c)). By 28 dah, the development of vertebrae in juvenile *M. miiuy* was completed, and no significant difference was observed in the biological morphology of vertebrae of juvenile *M. miiuy* of 28 dah compared with that of vertebrae of juvenile *M. miiuy* of 29 and 30 dah (Figure 1(d)).

3.2. Developmental Characteristics of Appendicular Skeleton

3.2.1. Developmental Characteristics of Paired Fins. The pectoral fins of *M. miiuy* were formed before the ventral fins. At three dah, the coracoid-scapula cartilages were clearly visible in larvae (Figure 2(a)). At 12 dah, the pectoral fin plate of larvae was clear with a fissure in the middle, and the cleithrum and supracleithrum were visible (Figure 2(b)). As the larvae grew and developed, the fin fold gradually widened. By the 21 dah, the fissures on the fin plate were increased to three, which were fan-shaped, and dozens of fin rays were clearly differentiated radially (Figure 2(c)). At 28 dah, the pectoral fin bone was completely developed, 21 fin rays in juveniles, and no significant morphological changes were observed between the juveniles at 28 and 27 dah (Figure 2(d)).

The development of ventral fins began with the appearance of cartilages of fin plate at 19 dah (Figure 3(a)). Five ventral fin rays were clearly visible in larvae at 21 dah when ossification also occurred in fin spines and rays (Figure 3(b)). Next, the fin spines and rays gradually ossified from the base to the distal end. Twenty-eight dah, the ossification was complete, with clear pelvic bones and fin rays (Figure 3(c)).

3.2.2. Developmental Characteristics of the Median Fin. The median fins of *M. miiuy* is formed in the order of caudal, dorsal and anal fins. Three hypural cartilaginous spines were formed at the ventral posterior notochord (Figure 4(a)). The hypural caudal fins differentiated further and increased to five at 14 dah, four of which were wide and flat. The last one was the smallest and rod-like with a tapered bottom. The gap between the second and third hypural fins was slighter larger than that between others. Moreover, there were 18 radial primitive fin rays, which indicated that the caudal fins tended to mature. The last caudal vertebra curved upward to form the urostyle, and three rod-like epural cartilaginous spines were clearly visible between the urostyle and medullary spine at third from bottom (Figure 4(b)). At 17 dah, the caudal fin rays were abjoint obviously and ossified in the direction from base to the distal end (Figure 4(c)). At 28 dah, the caudal fins were fully developed (Figure 4(d)).

The fin plate was formed from the middle part of the dorsal fin spine at 13 dah, and the numbers of dorsal fin plates were >20 by 14 dah (Figure 5(a)). At 17 dah, the radial primitive fin rays appeared (Figure 5(b)). The basipterygium was nearly fully developed, and the fin rays were ossified from the base to the distal end (Figure 5(c)). By 28 dah, the ossification was completed, indicating that the dorsal fin was fully developed (Figure 5(d)).

The fin plate and fin ray appeared at 14 dah (Figure 6(a)) and 17 dah (Figure 6(b)), respectively. Ossification began from base to the distal end with the development of fins. By 28 dah, ossification was complete and identical to that of adult individuals (Figure 6(c)).

3.3. Spinal Vertebrae Abnormalities. Four types of deformities were observed: spinal bulging, splitting of vertebrae, and supernumerary and bifurcated neural spines in vertebrae. Abnormalities primarily occurred at 13–28 dah.

The redundancy of spines was observed in the middle of spinal vertebrae both at 13 and 21 dah, with two spines appearing at adjacent or similar nodes (Figure 7(a)). Lordosis was observed at 18, 19, and 20 dah (Figure 7(b)), while kyphosis was observed at 21 dah (Figure 7(c)). Lordosis was featured with upward curvature, while kyphosis was featured with downward curvature when observed laterally. Vertebral deformity was found in the 16th node at 21 dah and in the 7th and 9th nodes at 26 dah. Notably, some bones of the vertebrae were split but not completely separated

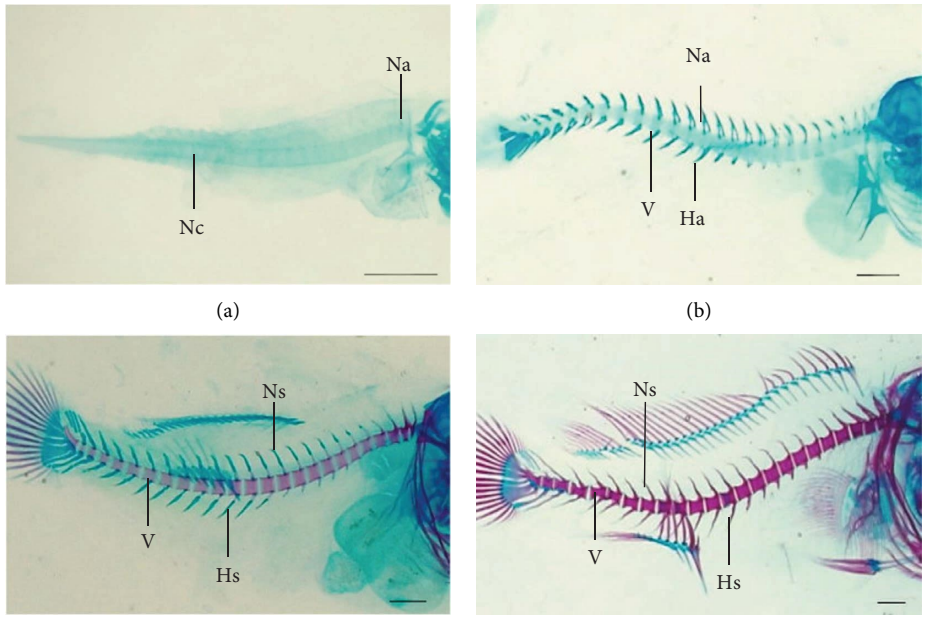

FIGURE 1: Vertebral column development timing and progression of larval and juvenile *Miichthys miiuy*. (a) 5 dah; (b) 12 dah; (c) 17 dah; (d) 28 dah. Nc: notochord; Na: neural arch; Ha: haemal arch; Ns: neural spine; Hs: haemal spine; *V*; vertebra. Bars = 1 mm.

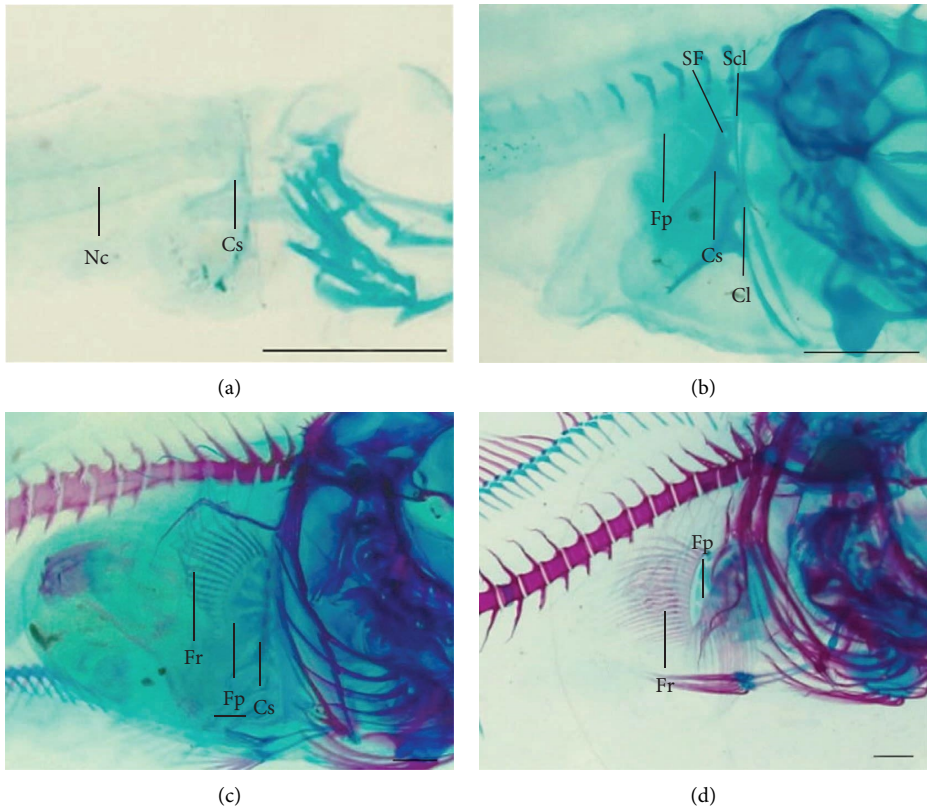

FIGURE 2: Pectoral fin development timing and progression of larval and juvenile *Miichthys miiuy*. (a) 3 dah; (b) 12 dah; (c) 21 dah; (d) 28 dah. Cl: cleithrum; CS: coracoid-scapula cartilage; Fp: fin plate; Fr: fin ray; Scl: supracleithrum; SF. scapula foramen. Bars = 1 mm.

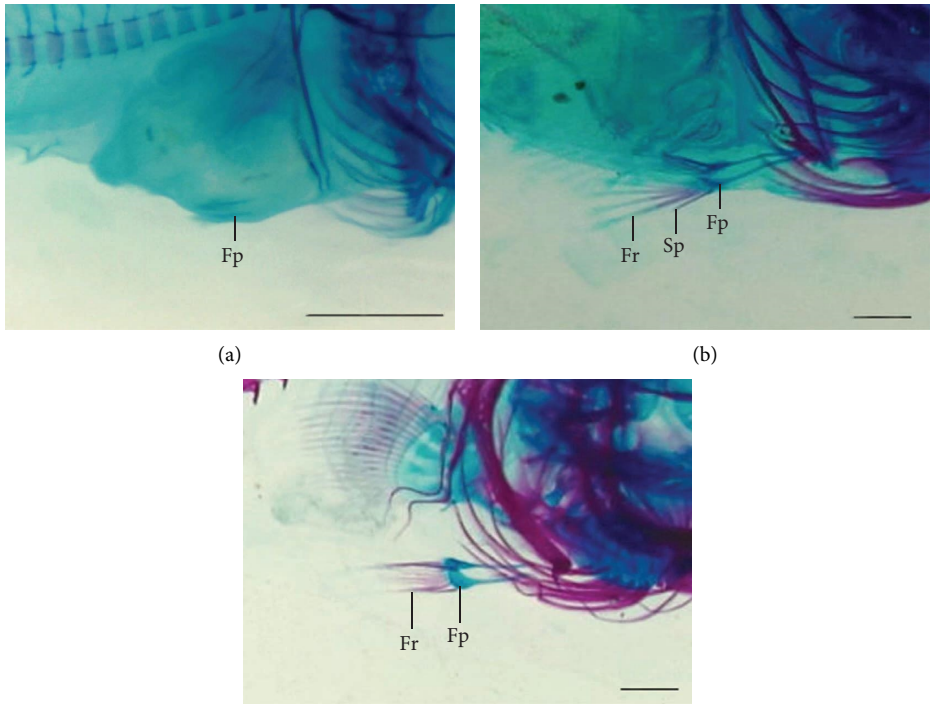

Figure 3: Ventral fin development timing and progression of larval and juvenile *Miichthys miiuy*. (a) 19 dah; (b) 21 dah; (c) 28 dah. Bars = 1 mm.

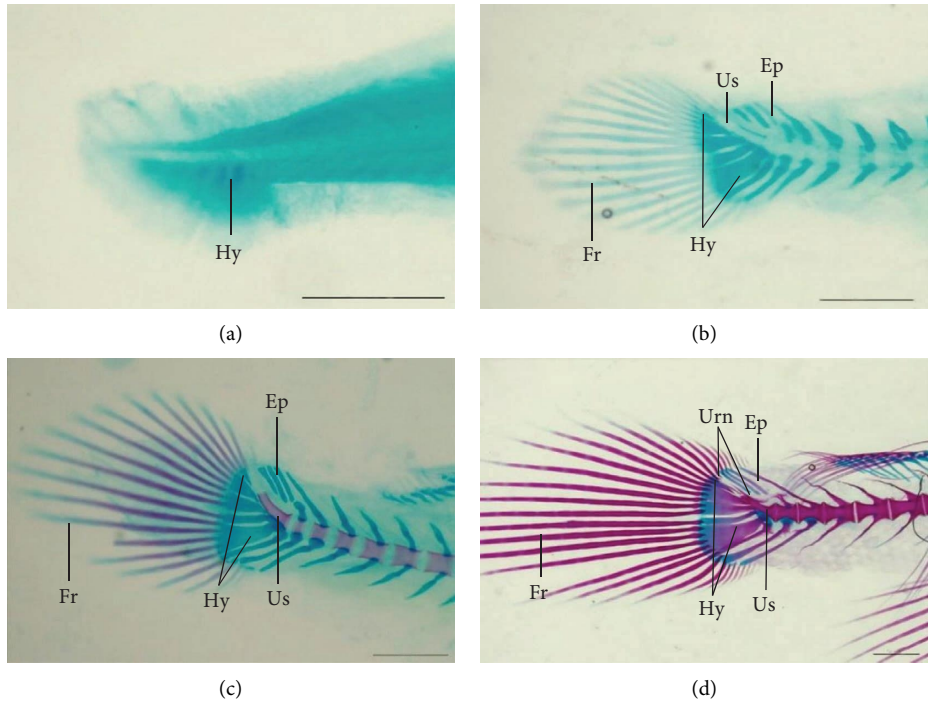

Figure 4: Caudal fin development timing and progression of larval and juvenile *Miichthys miiuy*. (a) 10 dah; (b) 14 dah; (c) 17 dah; (d) 28 dah. Hy: hypural; Ep: epural; Ur: urostyle; Urn: Uroneural. Bars = 1 mm.

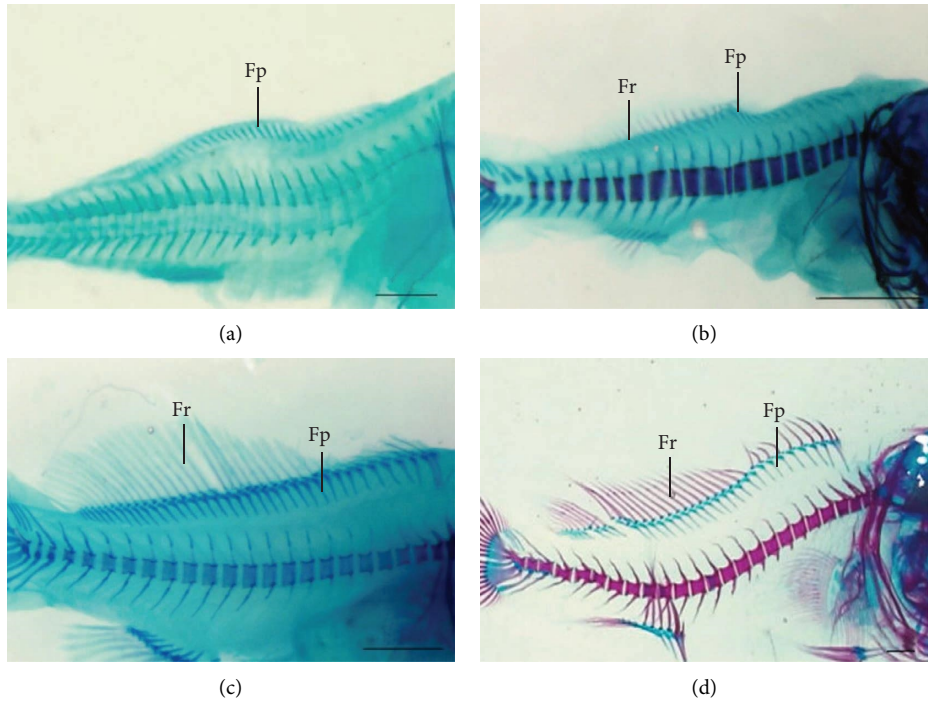

FIGURE 5: Dorsal fin development timing and progression of larval and juvenile *Miichthys miiuy*. (a) 14 dah; (b) 17 dah; (c) 21 dah; (d) 28 dah. Bars = 1 mm.

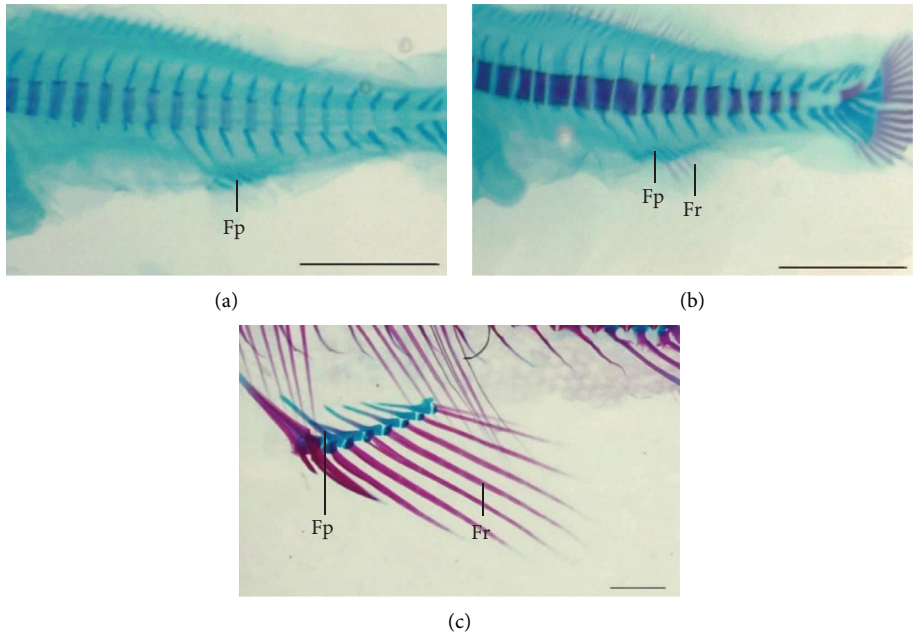

FIGURE 6: Anal fin development timing and progression of larval and juvenile *Miichthys miiuy*. (a) 14 dah; (b) 17 dah; (c) 28 dah. Bars = 1 mm.

(Figure 7(c)). The bifurcated neural spines were observed in 4th node at 21, 24, and 25 dah at the middle of the spine. The neural spine was divided into two parts from middle and upper part, with a dendritic bifurcation at the end (Figure 7(d)).

3.4. Skeletal Growth of Larvae and Juveniles. We found that the relationship between corresponding day-age (x_1) and skeletal length (y) was $y = 0.0032x_1^2 + 0.0971x_1 + 1.1353$ ($r^2 = 0.942$). The skeletal length of larvae at 1 dah was 1.63 ± 0.02 mm and reached 7.18 ± 0.52 mm after 29 days,

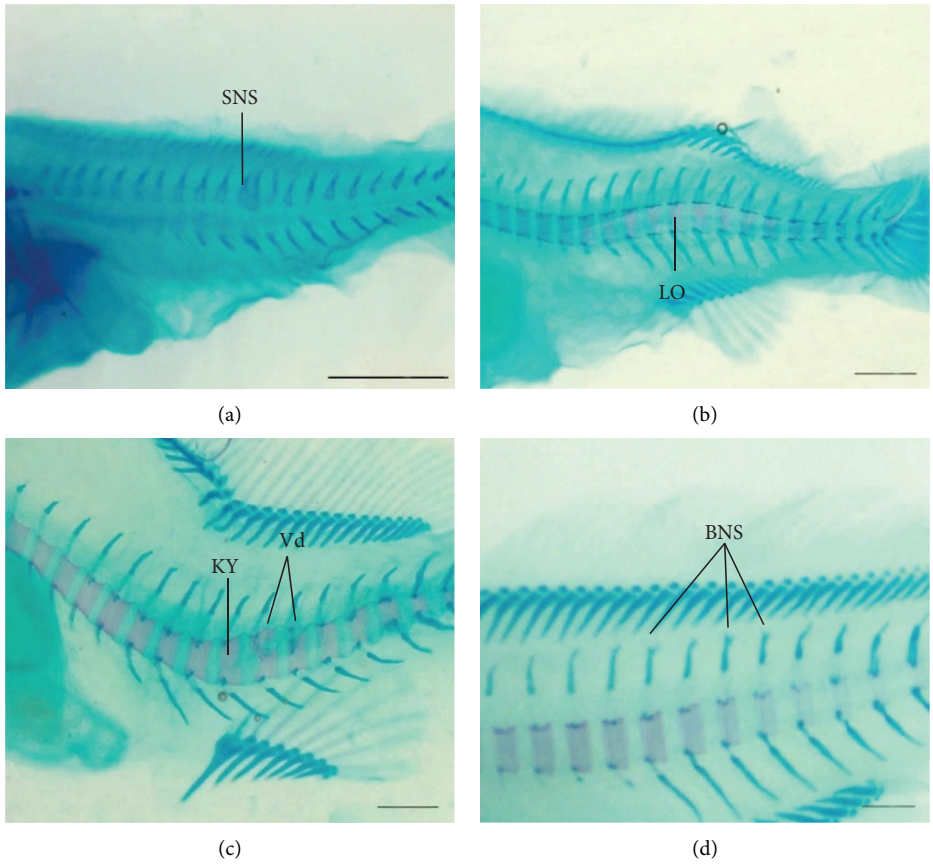

FIGURE 7: Vertebral column deformities of larval and juvenile *Miichthys miiuy*. (a) 13 dah; (b) 19 dah; (c) 21 dah; (d) 24 dah. SNS: supernumerary neural spines; BNS: bifurcated neural spines; LO: lordosis; KY: kyphosis; VD: vertebra deformity. Bars = 1 mm.

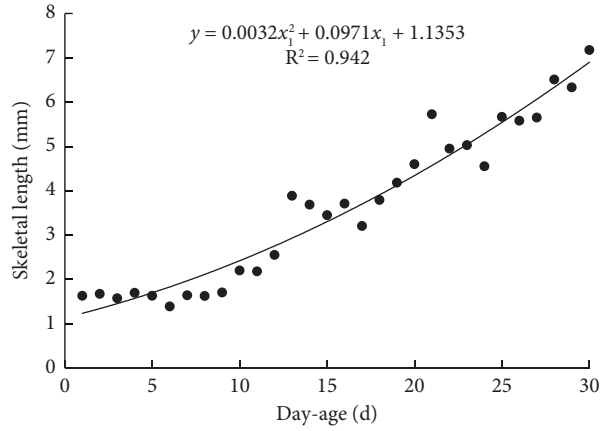

FIGURE 8: Relationship between skeleton length and day-age of the juvenile *Miichthys miiuy*.

with an average growth rate of 0.191 mm/d (Figure 8). The relationship between total length (x_2) and skeletal length (y) was $y = -0.0049{x_2}^2 + 0.5361x_2 + 0.2444$ ($r^2 = 0.979$; Figure 9). Additionally, the relationship between body length (x_3) and skeletal length (y) was $y = -0.002x_3^2 - 0.6506x_3 + 0.1465$ ($r^2 = 0.978$; Figure 10).

4. Discussion

4.1. Analysis of Developmental Characteristics on Spinal Vertebrae and Appendicular Skeleton. To date, many studies on skeletal developmental characteristics of larval and juvenile fish have been reported. For instance, the

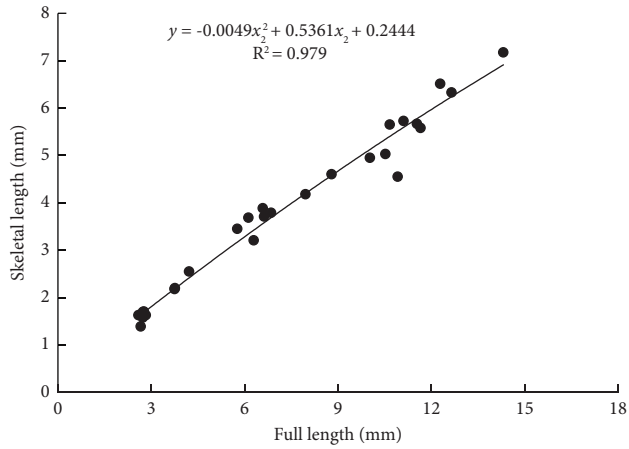

Figure 9: Relationship between skeleton length and full length of the juvenile *Miichthys miiuy*.

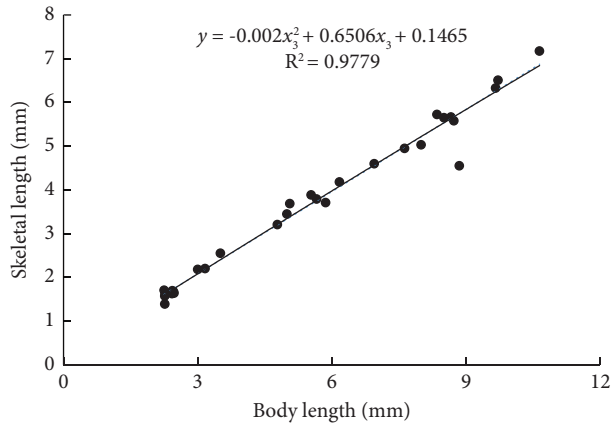

Figure 10: Relationship between skeleton length and body length of juvenile *Miichthys miiuy*.

developmental pattern of spinal vertebrae, pectoral fin, and caudal fin of the large yellow croaker (*Larimichthys crocea*) have been studied using the double staining method [17]. The results showed that the larval notochord appeared segment at 6 dah, and the neural and haemal archs developed sequentially. The vertebral cartilage was formed at 14 dah and ossified completely at 28 dah from the base to the distal end. The pectoral fin was the earliest to develop among the appendicular skeleton. The supracleithrum, cleithrum, and postcleithrum appeared at 3 dah, and proximal pterygrophores and fin rays developed completely at 27 dah. Further, the ossification was complete at 28 dah. The results of our study are consistent with those of Wang et al. [17]; likely because *L. crocea* and *M. miiuy* both belong to Sciaenidae and have a similar developmental sequence.

In a previous study, the early morphological development of *M. miiuy* was observed and analysed [18]. The results showed that the median fins were formed in the order of caudal, anal, and dorsal fins, and the appendicular skeleton was fully developed by 17 dah. All fins were completely differentiated at 28 dah, which also indicated the transformation of *M. miiuy* from larval to juvenile stage. The main reasons for these differences may be attributed to the differences in water temperature, light intensity, fish bait abundance, and feeding method during the breeding period, which may lead to a different developmental sequence in the different parts of larval and juvenile *M. miiuy*. Moreover, the difference in the developmental stages may be due to the use of different classifications.

The development of spinal vertebrae varies among different fish species. In our study, there were 25 spinal vertebrae in *M. miiuy*, which consist of 12 abdominal vertebrae and 13 coccyges. While Lates calcarifer have 24 spinal vertebrae during the developmental process of larvae and juveniles aged 0–23 days [19], euthynnus affinis have 20 trunk vertebrae and 18 tail vertebrae [20].

The development of the vertebral column and appendicular skeleton of the larvae and juveniles of Chaeturichthys stigmatias was also described [21]. The results revealed 40–43 spinal vertebrae, which consisted of 15 trunk vertebrae and 25–28 tail vertebrae. The number of spinal vertebrae had an important influence on biological characteristics like size, length, and height [22]. This data can provide a theoretical basis for the study of fish traits and biological classification through the analysis of developmental characteristics of fish vertebrae [23].

4.2. Reasons of Vertebral Column Deformities. The skeletal deformities can not only affect the movement, and external characteristics of fish but also their economic value [24]. The research development on skeletal deformities of larval and juvenile fish in recent years have been summarized by a recent review [25]. The results showed that skeletal deformities may cause poor food intake, poor growth, weak physical ability, and low survival, which may ultimately lead to large economic losses. Generally, common skeletal deformities in fish include lordosis, kyphosis, vertebrae ectopic, and neural and haemal spines deformities, among which the rate of vertebral deformity is the highest. The influencing factors of skeletal deformities include heredity, environment, and nutrition. Many studies have confirmed that proteins, lipids, and vitamins can affect the development of larval and juvenile fish to a certain extent. Notably, vitamins have considerable effects on skeletal development. For example, vitamin D can promote cartilage formation. Further, vitamin C deficiency has been shown to increase the probability of developmental deformities such as lordosis and kyphosis in larvae and juvenile fish [26]. Several skeletal abnormal phenomena such as lordosis, kyphosis, neural and haemal spines deformities, and vertebrae split were observed in our study, among which the vertebrae split was relatively uncommon. These skeletal deformities may be related to the feed nutrients provided during the artificial cultivation of larval and juvenile *M. miiuy*. However, other factors such as environment and disease can not be excluded. The relationship between skeletal deformities and heredity has not been investigated in this study due to the lack of observation and analysis on parent skeleton of *M. miiuy*.

Data Availability

The data that support the findings of this study are available upon request from the corresponding author.

Ethical Approval

All procedures complied with ethic committee of Ningde Normal University regarding animal experimentation.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

F.-F. Z.: methodology, datacuration, writing-original draft, and visualization. W.-Q. H.: methodology, project administration. S. L. and L. Z.: methodology, data curation. X.-J. S.: project administration, review. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

This research was funded by Fujiang Provincial Industry-Academic Collaboration Innovation Foundation (No. 2021N5005); Bureau of Science and Technology of Ningde [20190010]; Scientific Research Foundation of Ningde Normal University (grant numbers: 2023FZ13 and 2017FZ04).

References

[1] D. Sfakianakis, G. Koumoundouros, P. Divanach, and M. Kentouri, "Osteological development of the vertebral column and of the fins in Pagellus erythrinus (L. 1758). Temperature effect on the developmental plasticity and morpho-anatomical abnormalities," *Aquaculture*, vol. 232, no. 1-4, pp. 407–424, 2004.

[2] Z. Xu, M. Y. Li, and H. Q. Chen, "Study on the embryonic development of miichthys miiuy," *Marine Sciences*, vol. 2, pp. 93–97, 2007.

[3] C. Boglione, F. Gagliardi, M. Scardi, and S. Cataudella, "Skeletal descriptors and quality assessment in larvae and post-larvae of wild-caught and hatchery-reared gilthead sea bream (*Sparus aurata* L 1758)," *Aquaculture*, vol. 192, pp. 1–22, 2001.

[4] W. Q. Huang, Y. Zhang, F. F. Zhou et al., "Vertebrae of a large yellow croaker (larimichthys crocea) along with its early development," *Marine Sciences*, vol. 45, no. 2, pp. 51–58, 2021.

[5] F. F. Mao, *Genome-wide Identification and Expressionprofiling of the Bone Morphogenetic Proteins Gene Family, Osteologicalontogeny in Larval and Juvenile Rachycentron canadum*, Guangdong Ocean University, Guangzhou, China, 2021.

[6] B. Han, *Morphological Characters of Nibea Albiflora and Miichthys Miiuy and Genetic Research of Nibea Albiflora Populations*, Ocean University of China, Qingdao, China, 2007.

[7] H. Gou, *Biogenic Amines Profile and Characteristic Amines Analysis at Differene Storage Temperature in marine Fishes*, Zhejiang university, Hangzhou, China, 2015.

[8] B. Lou, "Fishery biology and artificial breeding techniques of miichthys miiuy," *Fishery Modernization*, vol. 6, pp. 11–13, 2004.

[9] X. Q. Chen, H. S. Zhan, and J. F. Shen, "Culture of miichthys miiuy in marine cage," *Ocean and Fishery*, vol. 7, pp. 60–62, 2017.

[10] S. L. Sun, X. Li, and X. H. Gu, "Analysis and evaluation of nutrient compositions of miichthys miiuy muscle and by products," *Food Machiner*, vol. 36, pp. 45–49, 2020.

[11] R. X. Zhou, *Characterization,function and Regulation of DIGIRR Gene in Miiuy Croaker*, Zhejiang Ocean University, Zhoushan, China, 2020.

[12] Y. Shen, *Relation between Main Miichthys Miiuy Rotten Grams and Corrupt and Study of Preservative Technology*, South China Agricultural University, Guangzhou, China, 2017.

[13] S. S. Gai, S. G. Guan, C. Y. Yu et al., "Skeletal deformities in larvae and juveniles of hatchery reared greenling hexagrammos otaki," *Journal of dalian ocean university*, vol. 35, no. 4, pp. 544–550, 2020.

[14] X. J. Shan and S. Z. Dou, "Allometric growth of croaker miichthys miiuy larvae and juveniles and its ecological implication," *Oceanologia et Limnologia Sinica*, vol. 40, no. 6, pp. 714–719, 2009.

[15] S. L. Zhao, Q. P. Lian, and J. S. Zhong, "Junsheng tudy on the skeleton development in relation to their feeding habit in miichthys miiuy larvae," *Journal of Fisheries of China*, vol. 32, no. 5, pp. 704–710, 2008.

[16] P. P. Deng, Y. H. Shi, J. B. Xu et al., "Early development of the vertebral column and appendicular skeleton of *Alosa*

sapidissima," *Journal of Fishery Sciences of China*, vol. 24, no. 1, pp. 73–81, 2017.

[17] Q. R. Wang, Y. Y. Ni, L. M. Lin, and Z. Y. Wang, "Development of the vertebral column and the pectoral and caudal fins in larvae of the large yellow croaker larimichthys crocea (richardson)," *Acta Hydrobiologica Sinica*, vol. 36, no. 3, pp. 467–472, 2010.

[18] Q. H. Shen, W. D. Shi, and J. Z. Sun, "Morphological studies on the early development of miichthys miiuy," *South China Fisheries Science*, vol. 1, no. 6, pp. 8–17, 2005.

[19] S. J. Zhou, T. Ma, J. Hu et al., "Skeletal deformities in larvae and juveniles of hatchery reared greenling hexagrammos otakii," *Journal of southern Agriculture*, vol. 49, no. 3, pp. 592–598, 2018.

[20] S. J. Zhou, R. Yang, G. Yu, Y. T. Liu, Z. H. Ma, and M. A. Zhenhua, "Description of euthynnus affinis vertebrae and appendages," *South China Fisheries Science*, vol. 18, no. 1, pp. 84–89, 2022.

[21] J. L. Huang, F. Hu, X. J. Song, Y. G. Chen, and J. S. Zhen, "Development of the vertebral column and appendicular skeleton in larvae and juveniles of chaeturichthys stigmatias," *Journal of Shanghai Ocean University*, vol. 31, no. 1, pp. 71–85, 2022.

[22] Y. Wang, J. J. Zhao, Q. Z. Ke et al., "Comparison of bone microstructures in cultured and wild stocks of large yellow croaker (larimichthys crocea) using micro CT scanning," *Marine Sciences*, vol. 40, no. 12, pp. 36–40, 2016.

[23] C. Y. Yu, S. G. Guan, D. D. Yu et al., "Development of vertebral column and appendicular skeleton in lavae and juveniles of fat greenling *Hexagrammos otakii*," *Journal of Dalian Oceanography*, vol. 35, no. 1, pp. 47–55, 2020.

[24] H. Ma, Z. M. Zhuang, S. F. Liu, Q. Ma, and X. L. Wang, "Skeletal deformities in the larvae and juveniles of cultured tongue sole (cynoglossus semilaevis)," *Journal of Fishery Sciences of China*, vol. 18, no. 6, pp. 1399–1405, 2013.

[25] M. Y. Han, S. J. Zhou, and Z. H. Ma, "Progress on skeletal ontogeny and deformities of fish larvae and juveniles: a review," *Chinese Journal of Fisheries*, vol. 34, no. 2, pp. 94–100, 2021.

[26] I. Ebi, A. S. K. Yong, L. S. Lim, and R. Shapawi, "Dietary ascorbic acid requirement for the optimum growth performances and normal skeletal development in juvenile hybrid grouper, epinephelus fuscoguttatus Epinephel us lanceolatus," *Journal of King Saud University Science*, vol. 30, no. 4, pp. 493–499, 2018.