Osteological Development of the Vertebral Column and Caudal Complex in Larval and Juvenile Blackhead Seabream, *Acanthopagrus schlegelii* (Perciformes, Sparidae) (Bleeker, 1854)

Jing Hu[1,2], Yan Liu[2,3], Gang Yu[1,2], Changping Yang[2,3], Binbin Shan[2,3], Shengnan Liu[2,4] and Dianrong Sun[2,3],*

[1]*South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Sanya 572018, China*

[2]*Key Laboratory of South China Sea Fishery Resources Exploitation and Utilization, Ministry of Agriculture, Guangzhou, 510300, China*

[3]*Key Laboratory of Fishery Ecology and Environment, Guangdong, Guangzhou, 510300, China*

[4]*Tianjin Agriculture Unitersity, Tianjin 300000, China*

Jing Hu and Yan Liu contributed equally to this study and share first authorship.

ABSTRACT

Osteological development of the vertebral column and caudal complex in blackhead seabream was described under extensive larval rearing conditions from 1DPH to 36DPH. The development of vertebral column originated from the occurrence of neural arch and hypural on 5DPH (SL2.4-3.4mmm, NL1.8-2.4mm), then skeletal ossification and Segmental bone formation occurred on 11DPH (SL3.6-4.0mm, NL2.6-2.8mm) and finished on 21DPH (SL9.6-11.6mm, NL7.4-8.6mm); Neural arch developed earlier than haemal arch, while haemal spine earlier than neural spine, consistent with the ossification; The development and ossification of neural arch and neural spine occurred in the backward order, while haemal arch and haemal spine from central tail to bothsides, What's more, all ossification occurred from proximal to outward; Along with the development of vertebral column, urostyle curved on 15-17DPH(SL4.0-5.0mm, NL2.9-3.4mm), then finished on 21DPH(SL4.6-5.2mm, NL3.4-3.8mm). During this progress, individuals present strong vitality of life and active action. This study explored the morphological characteristics of vertebral column and caudal skeletal ontogeny in larval *A. schlegelii,* which provides fundamental information for future artificial breeding and taxonomy of *A. schlegelii* and other relative species.

INTRODUCTION

The blackhead seabream *Acanthopagrus schlegelii* as a typical commercially valuable recreation and aquaculture fishery species is a marine teleost belonging to the perciform family Sparidae and widely distributed in the West Pacific Coasts from Japan, Korea to the East China Sea (Jean *et al*., 1996). For the delicious taste and high nutritional value, it has become a major marine-cultured species in China and other areas of Asia (Jean *et al*., 1998). Many researches are focus on the artificial breeding, diseases, nutriology and molecular biology of *A. schlegelii*, no report of early development of skeleton (Ho and Lin, 2005; Ho *et al*., 2007; Jeong *et al*., 2007; Ngandzali *et al*., 2011; Jin *et al*., 2017).

The skeletal system of fish is necessary to the survivor and growth of individuals, as it play an important in protecting internal organs, supporting the body and connecting muscle, also the source of motivation for feeding and avoiding the predators (Kohno *et al*., 1983, 1996; Dasilao and Yamaoka, 1998). Skeletal development is closely correlated with changes in swimming mode and also supports the anatomical and functional needs (Matsuoka, 1997; Koumoundouros and Sfakianakis, 2001; Cheng *et al.* 2018). In general, early skeletal development of *A. schlegelii* is the key to its motor function, avoiding predators and feeding success. Comprehensive knowledge of the osteological development of fish not only provides the means for understanding relationship of relative species and the fundamental data base for species identification,

---

but it also encourages the understanding of functional development and determines the conditions compatible with animal welfare (Koumoundouros and Sfakianakis, 2001; Huang *et al*., 2014). The osteological development in early stage closely relate with survivor of fish, as skeletal deformity leaded by unfavorable surrounding and poor nutrition will induce mass mortality, even if several survive, lack of market competitiveness with inferior external morphology, result in low breeding efficiency automatically (Koumoundouros *et al*., 1997; Wittenrich *et al*., 2009; Boglione *et al*., 2013). So the key to the success of aquiculture is the understanding of osteological development of fish in early stage. Double-stained method (Dingerkus and Uhler, 1977) was used to investigate the osteological development of larval and juvenile *A. schlegelii*, include the development of the vertebral column and caudal complex, which provide a certain taxonomic basis and contribute to the understanding of the functional trends and biological requirements in each developmental stage of individuals. This study will provide the foundational scientific basis of skeletal development of the Sparidae, and establishes a theoretical foundation for efficient aquaculture.

MATERIALS AND METHODS

Larval and juvenile rearing

Fertilized eggs of blackhead seabream were obtained from spawning tank through natural fertilization in a commercial fish hatchery, tropical Aquaculture Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Hainan Province, P.R. China, and the experiment was conducted in situ. Eggs were hatched in 500L fiberglass incubators at 27.5°C On 2 days post-hatch (DPH), larvae were reared into three 2500L tanks at a density of 60 fish·L^{-1}. Each rearing tank was supplied with filtered seawater (5µm pores) with a daily water exchange rate of 200% of the tank volume. Daily photoperiod of 14 h light and 10 h dark with 2000 lux light intensity was maintained at the water surface. The water temperature, salinity, PH, and dissolved oxygen were maintained at 29.0±1.0°C, 33 ± 0.8 ppt, 7.8-8.1 and 6.5 ± 0.2 mg L^{-1}, respectively. Total ammonia nitrogen < 0.1 mg L^{-1}, and nitrite nitrogen < 0.05 mg L^{-1}.

In the following feeding trials, the experimental condition was kept same as follows and any environmental modification was specified in each trial. One air stone was used in each bucket to maintain dissolved oxygen to near saturation. Fish were fed with rotifers (*Brachionus rotundiformis*) from 2 DPH to 9 DPH (SL 4.50±0.10mm) at a density of 10-20 rotifers mL^{-1}. On 7 DPH, *Artemia*

nauplii were introduced for co-feeding at 20-30nauplii mL^{-1} with *B. rotundiformis* deing contrast till 17DPH. Afterwards, *B. rotundiformis* were gradually replaced by *Artemia salina* from 18 DPH at 50-60 mL^{-1} *Artemia salina* with contrasting *Artemia* nauplii till the end of experiment. Live foods were enriched with the DHA protein Selco (INVE Aquaculture, Salt Lake City, UT, USA) for 12hours before feeding. To sure significant food and green surrounding, *Nannocholoropsis* sp. was induced during feeding of rotifer. Filters were cleaned to prevent outlet were choked up and siphon method was used to eliminate excrement, residue and dead organism daily.

Observations and measurements

A total of 20 samples were collected from rearing tankson1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 17, 19, 21 and 24 DPH respectively, and then anaesthetized with Aqui-S (AQUI-S New Zealand Ltd., Lower Hutt, New Zealand) at 40mgL^{-1}. The individuals were observed under microscopic to eliminate individuals with abnormal osteological development and measure standard and notochord length of normal ones. Then followed the protocol as following: initially fixed in 10% neutral buffered formalin, and then stained with alcian blue and alizarin red followed by Taylor and Van Dyke (1985). After staining, samples were photographed under stereomicroscope (Olympus SZ40) equipped with a digital camera (Oneplus, 2001). A total of 400 larvae and juveniles were stained and photographed to identify the development of the vertebral column and caudal complex. The terminologies of the skeleton were adopted from Kihara *et al*. (2002) and Sfakuanakis *et al*. (2004) and classification of developmental stage followed Kendall *et al*. (1984).

RESULTS

Vertebral column

Including the urostyle, the number section of the vertebra is 24, as the support components of vertebral column (Fig. 1). The vertebral column was formed by the vertebral centra, the neural and haemal arches and spines, the parapophyses and the ventral ribs. At hatching and in early ages of larval *A. schlegelii*, no metamerisms were observed (Fig. 1A-C). In this stage, the straight notochord was the only visible support structure that extended through the body at that period (1DPH, yolk-sac developmental stage, SL2.0-3.0mm, NL1.5-1.9mm, Fig. 1A). The first elements to be seen in the vertebral column are the neural and hypurals processes, which start to develop on 5-7 DPH (SL2.4-3.4mm, NL1.8-2.4mm). Their formation takes place via two developmental centers, front-end of notochord and middle part of caudal, respectively.
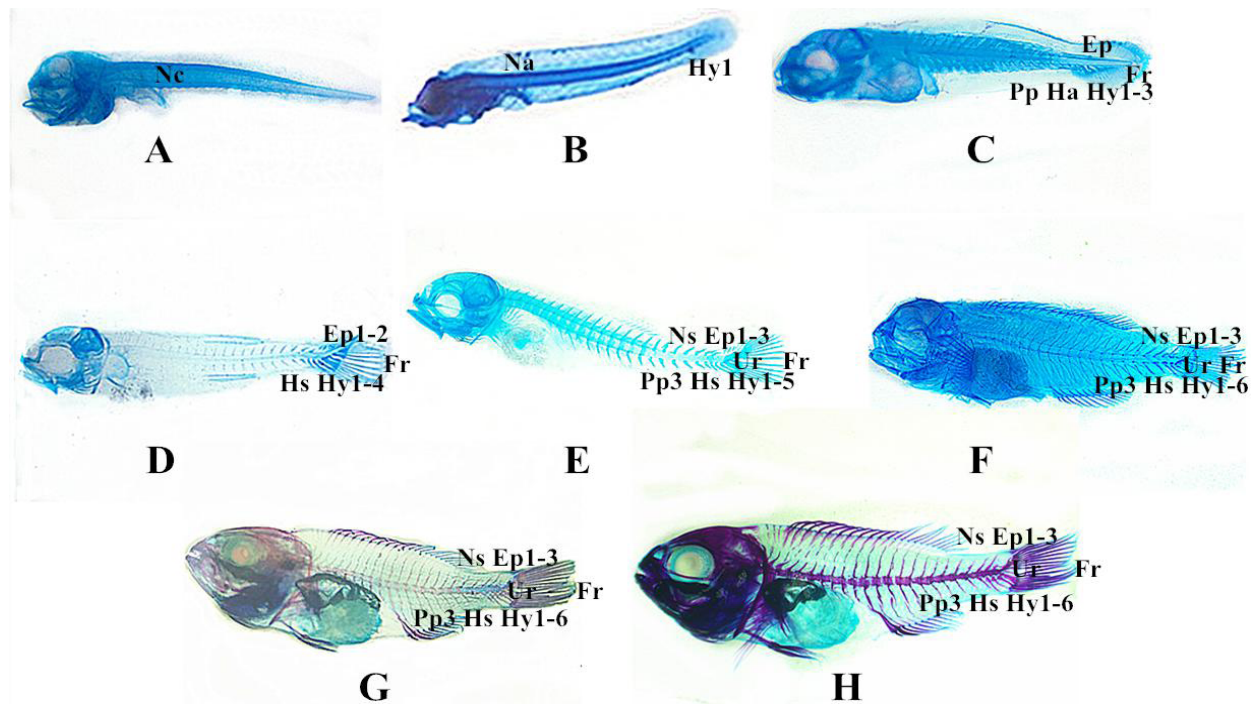
Fig. 1. Ontogeny of blackhead seabream vertebral column (A, 1DPH; B, 5-7DPH; C, 9DPH; D, 11DPH; E, 13DPH; F, 17DPH; G, 21DPH; H, 24DPH). Nc, notochord; Na, neural arch; Hy, hypural; Ep, epural; Pp, parapophysis; Ha, haemal arch; Fr, fin rays; Hs, haemal spine; Ns, neural spine; Ur, urostile.

Each neural arch is composed of a pair of cartilage, located on both sides of the center of notochord back. The formation and ossification of neural arch both occurred backwards (Fig. 1B). On 9DPH (SL3.2-3.8mm, NL2.4-2.6mm, Fig. 1C), haemal arch was observed, and the process of formation occurred from the central part of caudal to anterior and posterior. Ossified parapophysis present through a budding process. At the same time, Ossified haemal spine occurred on the next few ventralis vertebra of vertebral body where parapophysis occurred. On 11DPH (SL3.6-4mm, NL2.6-2.8mm, Fig. 1D), inter-segmental vertebrae occurred, Ossified haemal spine and neural spine increased. Cartilaginous neural arch began to modified, and ossification of vertebral column processed in the same direction of neural arch. On 13DPH (SL4.2-5.0mm, NL3.0-3.4mm, Fig. 1E), the ossification process reach to the caudal end, and the three ossified parapophysis occurred at the time. The upward flexion of the urostyle significantly and the caudal rays started to ossify within the primordial fin, in the area adjacent to the cartilaginous hypurals. Till 17DPH (SL4.6-5.2mm, NL3.4-3.8mm, Fig. 1F), major vertebral body formed, except to the modified neural spine in the caudal region, the formation of other elements of vertebral column, as vertebral body, haemal spine and neural spine all are finished. The upward flexion

of the urostyle process further to the angle of 30, and began to ossify in partial. On 21DPH (SL9.6-11.6mm, NL7.4-8.6mm, Fig. 1G), expect to the end of the urostyle, the ossification of vertebral column finished completely, the urostyle stop to curve anymore, all vertebral body formed in this stage. On 24 DPH (SL14.2-16.1mm, NL9.8-10.7mm, Fig. 1H), the ossification of vertebral body was finished and connect one by one, then the anterior and posterior apophyse occurred. The skeleton formed basically in this stage. During the development, neural arch developed and ossified earlier than haemal arch, while haemal spine earlier than neural spine, all ossification occurred from proximal to outward.

Caudal complex

The first four days after hatching, no development of caudal skeleton occurred, without caudal plates and fin rays, and the upwarp of urostyle also haven't happened this time (Fig. 2A). About 5DPH (SL2.3-3.3mm, NL1.7-2.3mm, Fig. 2B), as the developmental origin of caudal skeleton, the occurrence of cartilaginous hypurals located (Hy1)on veutro of after-end of notochord consider as the first appearance of caudal skeleton. About 7 DPH (SL2.4-3.4mm, NL1.8-2.4mm, Fig. 2C), Hy2 occurred by the offside of Hy1, and parhypural (Phy) began to differentiate

and emerge. On 9DPH (SL3.2-3.8mm, NL2.4-2.6mm, Fig. 2D), with the appearance of five fin rays, Hy3 occurred by the offside of Hy2, and the first epural appeared right above the Hy1 through a budding process. Phy elongated, and cartilaginous haemal spine occurred by the left side. On 11-13DPH (SL3.6-5.0mm, NL2.6-3.4-2.8mm, Fig. 2E, F), Hy4, Hy5 successively occurred rightward. Incompletely ossified neural spine (NS) occurred in the upper section of middle part caudal region (Fig. 2E). Hy1-Hy3, Expand Phy enlarged significantly with fuzzy edge between each other, and the tail began to divide into two parts with the Ur curve upward significantly (Fig. 2E). In this stage, ossification of notochord in the caudal region hasn't observed, and the fin ray number increase to 10. Completely ossified fin rays attached to the terminal of four hypurals. On 15-17DPH (SL4.0-5.2mm, NL2.9-3.8mm, Fig. 2G), the 6th hypural (Hy6) was observed. The Hy1-Hy5, Hs, Ns and Ep enlarge further with clearer edge. The number of fin rays increased to 14 and the notochord ossified to the front-end of CA. On 21 DPH (SL9.6-11.6mm, NL7.4-8.6mm, Fig. 2H), the process continue to conduct to the CA and the Ur ossified partly with maximum bending Angle. The whole CA was expanded and original caudal fork was observed. The intersegmental caudal fin rays began to ossify as the

rudiment of caudal skeleton formed. In this stage, 17 fin rays ossified partly and attach to the terminal of Ep, Hy and Phy. The ossification process of notochord extended to Hy6, and Hy1-Hy6, Ep, Hs, Ns enlarged further with clear edge, as Hs and sectional hypurals began to ossify also. On and after 24 DPH (SL14.2-16.2mm, NL9.8-10.7mm, Fig. 2I), no significant alteration was observed of the caudal complex formation, the differences only focus on the degree of ossification of Ur, caudal fin plate and fin rays.

DISCUSSION

Adaptability and peculiarity of the vertebral column of blackhead seabream

With the development of technology of shaping and observing animal skeleton specimen, conventional anatomy, histology, scanning technique, x-ray fluoroscopy and staining were common used (Roo *et al.*, 2010; Cobcroft and Battaglene, 2013). Depend on the perfection of relevant technology, the study of fish skeleton improved consequently. As the advantage of low coat, easy operation, effective result and high-quality preserved sample, staining became the most popular method for study of skeletal ontogeny as double-stained method used in this study.
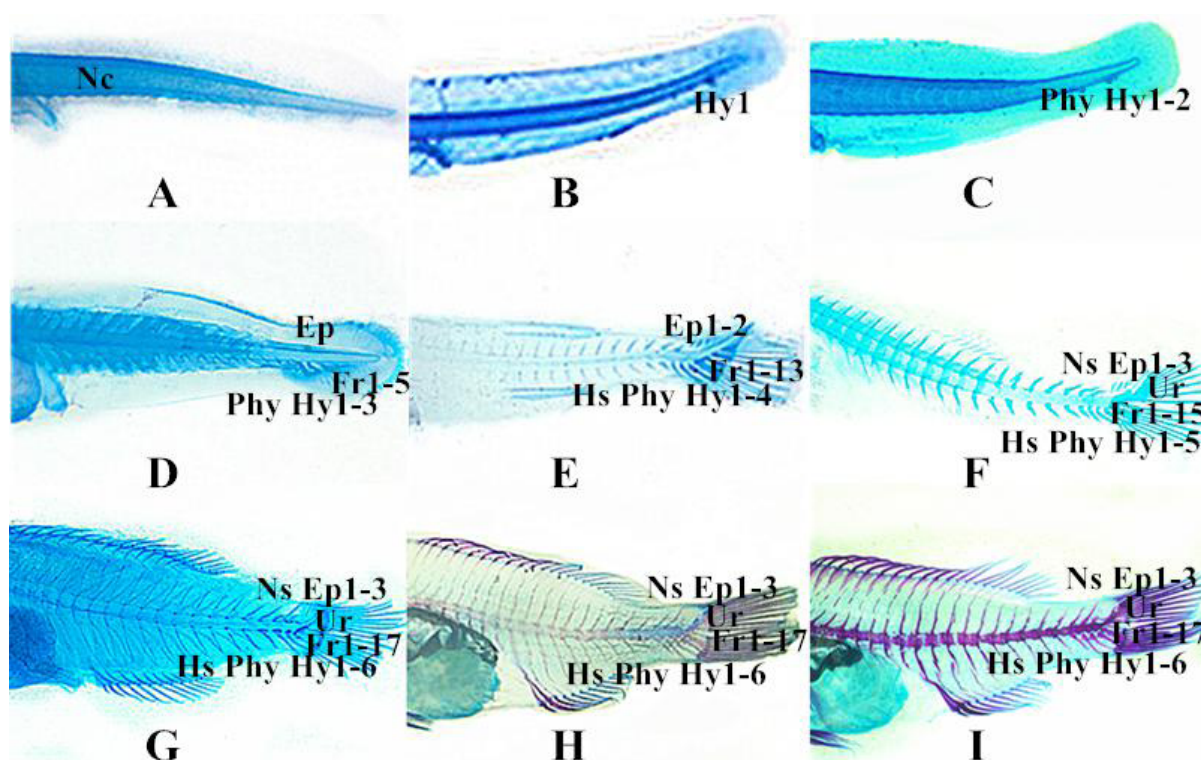

Fig. 2. Ontogeny of blackhead seabreamcaudal complex (A, 4DPH; B, 5DPH; C, 7 DPH; D, 9DPH; E, 11DPH; F, 13DPH; G, 15-17DPH; H:21DPH; I, 24DPH). Nc, notochord; Hy, hypural; Phy, parhypural; Ep, epural; Fr, fin rays; Hs, haemal spine; Ur, urostile; Ns, neural spine.

The difference of fish skeletal ontogeny is closely related to the environment and its own physiological function (Kohno *et al*., 1996). For example, the skeleton of Japanese rice fish (O*ryzias latipes*) (Langille and Hall, 1987) has begun to develop before hatch for the demand of swimming and feeding in larval stage; the vertebral column and caudal complex of Senegalese sole (*Solea senegalensis*) begin to develop on 12-13 DPH to adapt to benthic life (Gavaia *et al*., 2002). The study of fish skeletal ontogeny is one of the effective ways to grasp the relationship between structures and function (Fukuhara, 1992), and the understanding of relationship between skeletal ontogeny and the breeding environment can guide the artificial culture work effectively (Boglione *et al*., 2001). The skeletal ontogeny of Osteichthyes has a polymorphic development, and the development of various genera is much of a muchness. The skeletal ontogeny of the *O. latipes* (Langille and Hall, 1987) and *Inimicus japonicas* (Imamura and Yabe, 1997) and Salmonidae fishes begin to develop before hatch. Unlike these, the skeletal ontogeny of the *A. schlegelii* developed after hatching, which similar to that of the *Trachinotus ovatu* (Ma *et al*., 2014) and most of the Sparidae fishes (Doi *et al*., 2004). Similar to most Osteichthyes, the development of vertebral column of the *A. schlegelii* begins at the development of the hypura and neural, which was first found at 5DPH (SL2.4-3.4mm, NL1.8-2.4mm), while the developmental starting point of *T. ovatus* (Ma *et al*., 2014), *I. japonicas* (Imamura and Yabe, 1997), *Siniperca chuatsi* (Kendall *et al*., 1984), *Coilia nasus* (Chen *et al*., 2011) and *Seriola dumerili* (Laggis *et al*., 2010) are 7-9DPH (SL3.49-4.0mm), 8DPH (TL5.7mm), 6.4 mmNL, 11.5 mmNL and 4.6 mmSL, respectively. So, through the comparison, the development of vertebral column of *A. schlegelii* presents certain superiority in timing, as provides a basis for supporting the body and the further development of the feeding apparatus, and proved the relevant conclusion of rapid development and strong adaptability. The ossification process of vertebral column and the formation of intersegmental vertebrae occurred on 13 DPH (SL3.6-4.0mm, NL2.6-2.8mm), while the same process of *T. ovatus* (Ma *et al*., 2014), *S. chuatsi* (Kendall *et al*., 1984) and *S. dumerili* (Laggis *et al*., 2010) occurred on 11DPH, 11.5mmNL and 4.8mmNL (notochord length). Thus, from the aspect of ossification of vertebral column, the superiority in early development of *A. schlegelii* was proved further. Neural arch developed earlier than haemal arch, while haemal spine earlier than neural spine, consistent with the ossification; The same as *T. ovatus* (Ma *et al*., 2014), the development and ossification of neural arch and neural spine occurred in the backward order,

while haemal arch and haemal spine from central tail to bothsides, What's more, all ossification occurred from proximal to outward. Different from *S. chuatsi* (Kendall *et al*., 1984) as the formation and ossification of haemal arch and haemal spine both occurred in the backward order, while the ossification of neural arch and neural spine of *I. japonicas* (Imamura and Yabe, 1997) occurred from central part to proximal and outward. The formation and ossification of *C. nasus* are both different from *A. schlegelii*, as the ossification of haemal arch and haemal spine occurred in the backward order, and the ossification of each neural arch, neural spine, haemal arch and haemal spine occurred from central part to proximal and outward, similar to the *I. japonicas* (Imamura and Yabe, 1997). The above comparison results indicate that the understanding of skeletal ontogeny possess some taxonomic significance, confirm the inference that, as a kind of inferior fish, the *C. nasus* maybe a transitional species in the evolutionary process to a certain extent by Chen *et al*. (2011). The completion of the ossification process of vertebral column in *A. schlegelii* occurred on 24DPH (SL9.6-11.6mm, NL7.4-8.6mm), a little slower than *T. ovatus* (Ma *et al*., 2014) (18DPH, SL9.12mm), presents the diversity and complexity of the vertebrae development of Osteichthyes. The occurrence of this phenomenon may be related to the breeding management or environmental factors in the later stage (Aragão *et al*., 2001; Ma *et al*., 2012; ZamboninoInfante *et al*., 1997), and the relevant research needs to be carried out further.

Adaptability and peculiarity of the caudal complex of blackhead seabream

As big family, perciformes occupies larger proportion of marine economy fish trade, while blackhead seabream, as a typical species of artificial breeding, enhancement and releasing, of which the research in recent years has become increasingly mature (Xue *et al*., 2008; Ngandzali *et al*., 2011; Shi *et al*., 2012). Caudal fin of perciformes is characterized by no more than 17 fin rays, in the research of *A. schlegelii*, the fin rays have reaching a maximum of 17 on 21DPH, indicating a strong adaptability (Kohno *et al*., 1996).

The caudal fin has the function of propulsion and steering. In view of its complex formation and development process, the strengthening of function must be closely coordinated with the spinal extremities to provide effective impetus (Ignacio *et al*., 2008). The development of the caudal of blackhead seabream is closely consistent with the development of the vertebral column. The development of the caudal fin is accompanied by the curvature of the notochord, and the upwarping of the urostile is defined as the sign of entering the juvenile stage (Takushima *et al*.,

2003), and the *A. schlegelii* presents a strong vitality and activity in this stage. The upwarping of *A. schlegelii* began on 15-17DPH (SL4.0-5.0mm, NL2.9-3.4mm), accompanied by the ossification of vertebral column and the formation of Segmental bone. The ossification process of urostile is completed on 21DPH (SL4.6-5.2mm, NL3.4-3.8mm), and all vertebral bodies have formed and the vertebral column ossified completely except urostile at this time. A high consistency and synchronicity was presented between vertebral column and urostile, providing a guarantee for the improvement of the related functions such as feeding and avoiding the predators. Based on the observation of caudal complex development, microscopic observations were conducted of blackhead seabream's internal organs. The results show that, functional gastric developed completely on 17-21DPH, as a marker of division of larval and juvenile (Stroband and Kroon, 1981), presents a high consistency with the result of skeletal ontogeny study. The caudal complex of *A. schlegelii* mainly consists of six different sizes of hypural, three epural and one uroneural. Including Sparidae species, studies on skeletal ontogeny of various marine fish have been carried out in large numbers (Sfakianakis *et al.*, 2005; Park *et al.*, 2016; Zheng *et al.*, 2016), as the results show that there is a certain difference in structure and developmental timing of caudal complex between species. Such as *T. ovatus* (Zheng *et al.*, 2016), *Sparus aurata* (Koumoundouros *et al.*, 1997), *Diplodus sargus* (Koumoundouros and Sfakianakis, 2001), *D. puntazzo* (Sfakianakis *et al.*, 2005), *Epinephelus akaara* (Park *et al.*, 2016) and *Dentex dentex* (Koumoundouros *et al.*, 2001), the caudal complex of which all consist of five hypural, three epural and one uroneural, all of them have one less hypural, as the foundation of motor ability and feeding power, and suggests a strong adaptability to some degree. Although the structure of hypural of blackhead seabream differs from many marine fishes, the developmental timing is consistent with other fish, all in the left and right way in the caudal. In this study, the first hypural occurred on 5DPH (SL2.3-3.3mm, NL1.7-2.3mm) and the last 15-17DPH (SL4.0-5.0mm, NL2.9-3.4mm). The first-feeding occurred in the yolk sac (1-2DPH, SL1.9-2.1 mm, NL1.3-1.8mm) of the blackhead seabream, which means that blackhead seabream is transitioned from endogenous nutrition to exogenous nutrition, and then goes through the transformation of larval to juvenile on 15-17DPH. The study found that, both based on the development of vertebral column and caudal complex can showed an advantage of adaptability to the environment, and present that blackhead seabream is superior to many marine fishes. This advantage provides physiological needs for feeding conversion and feeding of larval-juvenile fish, and the necessary guarantee for its survival and growth.

Taxonomic significance of the skeletal ontogeny of blackhead seabream

The research on the development of skeletal ontogeny not only has the economic benefit of farming, but also has great revelation for the classification of fish. The skeletal ontogeny of fishes varies greatly between species (Zheng *et al.*, 2016), and the morphological characteristics of the skeletal ontogeny are an important basis for studying the classification of fish systems. Yang and Li (2008) compared the *Parargyrops edita* and *S. latus*, obtained the results that some significant differences exist between this two species expect for some common characteristics of sparidae, can be used as a basis for the identification of interspecies. Wang (1989) studied a vast amount of information for Sparidae in China seas, and compared their skull morphology and tooth characteristics. The results suggest that *Sparus*, *Rhabdosargus*, *Evynnis* and *Parargyrops* have close kinship, while *Pagrosomus*, *Argyrops* and *Dentex* are closely related. While very different from the results obtained from Akazaki (1962). A large number of scholars (Akazaki, 1962; De *et al.*, 2001; Carpenter and Johnson, 2002; Day, 2002; Orrell *et al.*, 2002; Orrell and Carpenter, 2004; Chiba *et al.*, 2009) have conducted a taxonomic study of the Sparidae in various ways, including molecular biology, and there are great differences. Especially the study on phylogenetic relationship between *A. schlegelii* and *A. schlegelii czerskii*, there's no clear and exact conclusion yet, lack of research support in osteology. Many research in osteological development of Sparidae have conducted, mainly focus on the early development and artificial breeding (Koumoundouros *et al.*, 1997, 1999, 2001; Sfakianakis *et al.*, 2005). The research on the use of skeletal ontogeny in taxonomy is slightly weak, and the combination of the related classification techniques will be an effective way to master the classification of the Sparidae.

CONCLUSIONS

A comprehensive understanding of the skeletal development improve the breeding efficiency of larval and juvenile fish and provide some evidence for taxonomy. This study has explored the skeletal ontogeny in blackhead seabream. Results from the present study would be useful to understand functional morphology and larval aquaculture of marine teleosts, and would be helpful to the classification of the Sparidae.

ACKNOWLEDGMENTS

in Fishery Stock Enhancement in the Tonkin Gulf).

Statement of conflict of interest
   Authors have declared no conflict of interest.

REFERENCES

Akazaki, M., 1962. *Studies on the spariform fishes: Anatomy, phylogeny, ecology and taxonomy.* Kosugi Co. Ltd., Osaka, Japan, pp. 1-368.

Aragão, C., Conceição, L.E.C., Fyhn, H.J. and Dinis, M.T., 2001. Amino acid profiles of sea bream (*Sparus aurata*) and sole (*Soleas enegalensis*) during first feeding: Are currently used diets imbalanced in amino acids. *Eur. Aquacul. Soc.*, **30**: 32-35.

Boglione, C., Gagliardi, F., Scardi, M. and Cataudella, S., 2001. Skeletal descriptors and quality assessment in larvae and post-larvae of wild-caught and hatchery-reared gilthead sea bream (*Sparus aurata* L. 1758). *Aquaculture*, **192**: 1-22. https://doi.org/10.1016/S0044-8486(00)00446-4

Boglione, C., Gavaia, P., Koumoundouros, G., Gisbert, E., Moren, M., Fontagné, S. and Witten, P.E., 2013. Skeletal anomalies in reared European fish larvae and juveniles. Part 1: normal and anomalous skeletogenic processes. *Rev. Aquacul.*, **5**: S99-S120. https://doi.org/10.1111/raq.12016

Carpenter, K.E. and Johnson, G.D., 2002. A phylogeny of sparoid fishes (Perciformes, Percoidei) based on morphology. *Ichthyol. Res.*, **49**: 114-127. https://doi.org/10.1007/s102280200015

Chen, Y., Xia, D., Zhong, J., Zhao, S. and Zhang, Y., 2011. Development of the vertebral column and the appendicular skeleton in the larvae and juveniles of *Coilia nasus*. *J. Shanghai Ocean Univ.*, **20**: 217-223.

Cheng, D.C., Hassan, M.M., Ma, Z.H., Yang, Q.B. and Qin, J.G., 2018. Skeletal ontogeny and anomalies in larval and juvenile crimson snapper, *Lutjanus erythropterus* Bloch, 1790. *Pakistan J. Zool.,* **50**: 799-807. http://dx.doi.org/10.17582/journal.pjz/2018.50.3.799.807

Chiba, S.N., Iwatsuki, Y., Yoshino, T. and Hnzawa, N., 2009. Comprehensive phylogeny of the family Sparidae (Perciformes: Teleostei) inferred from mitochondrial gene analyses. *Genes Genet. Syst.*, **84**: 153-170. https://doi.org/10.1266/ggs.84.153

Cobcroft, J.M. and Battaglene, S.C., 2013. Skeletal malformations in Australian marine finfish hatcheries. *Aquaculture*, **396-399**: 51-58. https://doi.org/10.1016/j.aquaculture.2013.02.027

Dasilao, J.R. and Yamaoka, K., 1998. Development of the vertebral column and caudal complex in a flying fish, *Parexocoetus mento mento*. *Ichthyol. Res.*, **45**: 303-308. https://doi.org/10.1007/BF02673928

Day, J.D., 2002. Phylogenetic relationships of the Sparidae (Teleostei: Percoidei) and implications for convergent trophic evolution. *Biol. J. Linn. Soc.*, **76**: 269-301. https://doi.org/10.1111/j.1095-8312.2002.tb02088.x

De, I.R.H., Rejon, C.R., Rejon, M.R. and Garridoramos, M.A., 2001. The molecular phylogeny of the Sparidae (Pisces, Perciformes) based on two satellite DNA families. *Heredity*, **87**: 691-697. https://doi.org/10.1046/j.1365-2540.2001.00967.x

Dingerkus, G. and Uhler, D., 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technol.*, **52**: 229-232. https://doi.org/10.3109/10520297709116780

Doi, T., Aoyama, S. and Kinoshita, I., 2004. Ontogeny of the mandarin fish *Siniperca chuatsi* (Perciformes: Sinipercidae) reared in aquarium. *Ichthyol. Res.*, **51**: 337-342. https://doi.org/10.1007/s10228-004-0239-1

Fukuhara, O., 1992. Study on the development of functional morphology and behaviour of the larvae of eight commercially valuable teleost fishes. *Contrib. Fish. Res. Japan Sea Block*, **25**: 1-122.

Gavaia, P.J., Dinis, M.T. and Cancela, M.L., 2002. Osteological development and abnormalities of the vertebral column and caudal skeleton in larval and juvenile stages of hatchery-reared Senegal sole (*Solea senegalensis*). *Aquaculture*, **211**: 305-323. https://doi.org/10.1016/S0044-8486(02)00167-9

Ho, J.S., Cheng, Y.R. and Lin, C.L., 2007. The development of *Parabrachiella lata* (Song & Chen, 1976), a lernaeopodid copepod parasitic on the black porgy, *Acanthopagrus schlegelii* (Bleeker, 1854), of Taiwan. *Crustaceana*, **80**: 47-66. https://doi.org/10.1163/156854007779696505

Ho, J.S. and Lin, C.L., 2005. Three species of Naricolax ho, do & kasahara, 1983 (Copepoda, Bomolochidae) parasitic in the nasal cavities of marine fishes of Taiwan. *Crustaceana*, **78**: 599-619. https://doi.org/10.1163/156854005774318114

Huang, C., Tan, X.Y., Wu, K., Chen, Q.L., Zhuo, M.Q., Pan, Y.X. and Song, Y.F., 2014. Osteological development and anomalies in larval stage of hatchery-reared yellow catfish *Pelteobagrus fulvidraco*. *Aquacul. Res.*, **47**: 1125-1140. https://doi.org/10.1111/are.12568

Ignacio, F., Francisco, H., Juan, B.O.D., Yannis, K., Alicia, E., JoseLuis, Z.I. and Enric, G., 2008. Larval

performance and skeletal deformities in farmed gilthead sea bream (*Sparus aurata*) fed with graded levels of vitamin A enriched rotifers (*Brachionus plicatilis*). *Aquaculture*, **283**: 102-115. https://doi.org/10.1016/j.aquaculture.2008.06.037

Imamura, H. and Yabe, M., 1997. Osteological development of the lumpfish, *Inimicus japonicus* (Pisces: Synanceiidae). *Ichthyol. Res*., **45**: 53-67. https://doi.org/10.1007/BF02678575

Jean, C., Lee, S., Chen, C. and Hui, C., 1998. Variation in mitochondrial DNA sequences of black porgy, *Acanthopagrus schlegelii*, in the coastal waters of Taiwan. *Zool. Stud*., **37**:22-30.

Jean, C., Lee, S., Hui, C. and Chen, C., 1996. Genetic variation of black porgy, *Acanthopagrus schlegelii* (Perciformes: Sparidae) in the coastal waters of Taiwan. *Biochem. System. Ecol*., **24**: 211-219. https://doi.org/10.1016/0305-1978(96)00003-8

Jeong, D.S., Gonzalez, E.B., Morishima, K., Arai, K. and Umino, T., 2007. Parentage assignment of stocked black seabream *Acanthopagrus schlegelii* in Hiroshima Bay using microsatellite DNA markers. *Fish. Sci*., **73**: 823-830. https://doi.org/10.1111/j.1444-2906.2007.01402.x

Jin, M., Monroig, Ó., Lu, Y., Yuan, Y., Li, Y., Ding, L.Y., Tocher, D.R. and Zhou, Q.C., 2017. Dietary DHA/EPA ratio affected tissue fatty acid profiles, antioxidant capacity, hematological characteristics and expression of lipid-related genes but not growth in juvenile black seabream (*Acanthopagrus schlegelii*). *PLoS One*, **12**: e0176216. https://doi.org/10.1371/journal.pone.0176216

Kendall, A.W., Ahlstrom, E.H. and Moser, H.G., 1984. Early life history stages of fishes and their characters. In: *Ontogeny and systematics of fishes* (eds. H.G. Moser, W.J. Richards, D.M. Cohen, M.P. Fahay, A.W. Kendall and S.L. Richardson). American Society of Ichthyologists and Herpetologists, Special Publication No 1, Allen, Lawrence, KS, pp. 11-22.

Kihara, M., Ogata, S., Kawano, N., Kubota, I. and Yamaguchi, R., 2002. Lordosis induction in juvenile red sea bream, *Pagrus major*, by high swimming activity. *Aquaculture*, **212**: 149-158. https://doi.org/10.1016/S0044-8486(01)00871-7

Kohno, H., Ordonio-Aguilar, R., Ohno, A. and Taki, Y., 1996. Morphological aspects of feeding and improvement in feeding ability in early stage larvae of the milkfish, *Chanos chanos*. *Ichthyol. Res*., **43**: 133-140. https://doi.org/10.1007/BF02348236

Kohno, H., Ordonio-Aguilar, R. and Taki, Y., 1996. Osteological development of the feeding apparatus

in early stage larvae of the seabass, *Lates calcarifer*. Ichthyol. Res., **43**: 1. https://doi.org/10.1007/BF02589604

Kohno, H., Taki, Y., Ogasawara, Y., Shirojo, Y., Taketom, I. and Inoue, M., 1983. Development of swimming and feeding functions in larvae *Pagrus major*. *Japanese J. Ichthyol*., **3**: 47-59.

Koumoundouros, G., Divanach, P. and Kentouri, M., 1999. Osteological development of the vertebral column and of the caudal complex in *Dentex dentex*. *J. Fish Biol*., **54**: 424-436. https://doi.org/10.1006/jfbi.1998.0874

Koumoundouros, G., Divanach, P. and Kentouri, M., 2001. Osteological development of *Dentex dentex* (Osteichthyes: Sparidae): Dorsal, anal, paired fins and squamation. *Mar. Biol*., **138**: 399-406. https://doi.org/10.1007/s002270000460

Koumoundouros, G., Gagliardi, F., Divanach, P., Boglione, C., Cataudella, S. and Kentouri, M., 1997. Normal and abnormal osteological development of caudal fin in *Sparus aurata* L. fry. *Aquaculture*, **149**: 215-226. https://doi.org/10.1016/S0044-8486(96)01443-3

Koumoundouros, G. and Sfakianakis, D.G., 2001. Osteological development of the vertebral column and of the fins in *Diplodus sargus* (Teleostei: Perciformes: Sparidae). *Mar. Biol*., **139**: 853-862. https://doi.org/10.1007/s002270100645

Laggis, A., Sfakianakis, D.G., Divanach, P. and Kentouri, M., 2010. Ontogeny of the body skeleton in *Seriola dumerili* (Risso, 1810). *Italian J. Zool*., **77**: 303-315. https://doi.org/10.1080/11250000903170870

Langille, R.M. and Hall, B.K., 1987. Development of the head skeleton of the Japanese medaka, *Oryzias latipes* (Teleostei). *J. Morphol*., **193**: 135-158. https://doi.org/10.1002/jmor.1051930203

Matsuoka, M., 1997. Osteological development in the Japanese sardine, *Sardinops melanostictus*. *Ichthyol. Res*., **44**: 275-295. https://doi.org/10.1007/BF02678707

Ma, Z., Qin, J.G. and Nie, Z., 2012. Morphological changes of marine fish larvae and their nutrition need. In: *Larvae: Morphology, biology and life cycle*. Nova Science Publishers, Inc., New York, USA, pp. 1-20.

Ma, Z., Zheng, P., Guo, H., Zhang, N., Jaing, S., Zhang, D. and Qin, J., 2014. Jaw malformation of hatchery reared golden pompano *Trachinotus ovatus* (Linnaeus 1758) larvae. *Aquacul. Res*., **47**: 1141-1149. https://doi.org/10.1111/are.12569

Ngandzali, B., Zhou, F., Xiong, W., Shao, Q. and Xu, J., 2011. Effect of dietary replacement of fish meal by

soybean protein concentrate on growth performance and phosphorus discharging of juvenile black sea bream, *Acanthopagrus schlegelii. Aquacul. Nutr*., **17**: 526-535. https://doi.org/10.1111/j.1365-2095.2010.00835.x

Orrell, T.M. and Carpenter, K.E., 2004. A phylogeny of the fish family Sparidae (porgies) inferred from mitochondrial sequence data. *Mol. Phylogenet. Evolut*., **32**: 425-434. https://doi.org/10.1016/j.ympev.2004.01.012

Orrell, T.M., Carpenter, K.E., Musick, J.A. and Graves, J.E., 2002. A phylogenetic and biogeographic analysis of the Sparidae (Perciformes: Percoidei) based on cytochrome b sequences. *Copeia*, **3**: 618-631. https://doi.org/10.1643/0045-8511(2002)002[0618:PABAOT]2.0.CO;2

Park, J.Y., Han, K.H., Cho, J.K., Myeong, J.I. and Park, J.M., 2016. Early osteological development of larvae and juveniles in red spotted grouper, *Epinephelus akaara* (Pisces: Serranidae). *J. Korean Soc. Develop. Biol*., **20**: 87-101.

Roo, F.J., Socorro, J.A., Hernández-Cruz, C.M., Fernández-Palacios, H. and Izquierdo, M.S., 2010. Osteological development and occurrence of skeletal deformities in red porgy *Pagrus pagrus* (Linnaeus, 1758) larvae cultured under different rearing techniques. *J. Fish Biol*., **77**: 1309-1324. https://doi.org/10.1111/j.1095-8649.2010.02753.x

Sfakianakis, D.G., Doxa, C.K., Kouttouki, S., Koumoundouros, G., Maingot, E., Divanach, P. and Kentouri, M., 2005. Osteological development of the vertebral column and of the fins in *Diplodus puntazzo* (Cetti, 1777). *Aquaculture*, **250**: 36-46. https://doi.org/10.1016/j.aquaculture.2005.03.042

Sfakianakis, D.G., Koumoundouros, G., Divanach, P. and Kentouri, M., 2004. 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*, **232**: 407-424. https://doi.org/10.1016/j.aquaculture.2003.08.014

Shi, X.F., Su, Y.Q., Wang, J., Ding, S.X. and Mao, Y., 2012. Complete mitochondrial genome of black porgy *Acanthopagrus schlegelii* (Perciformes, Sparidae). *Mitochond. DNA*, **23**: 310-312. https://doi.org/10.3109/19401736.2012.683182

Stroband, H.W.J.A. and Kroon, A.G., 1981. The development of the stomach in *Clarias lazera* and the intestinal absorption of protein macromolecules. *Cell Tissue Res*., **215**: 397-415. https://doi.org/10.1007/BF00239123

Takushima, M., Nozaki, R., Kadomura, K., Yasumoto, S. and Soyano, K., 2003. Induced ovulation using LHRHa and artificial fertilization in devil stinger, *Inimicus japonicas*. *Fish Physiol. Biochem*., **28**: 521-522. https://doi.org/10.1023/B:FISH.0000030650.24741.58

Taylor, W. and Van Dyke, G., 1985. Revised clearing and staining methods. *Cybium,* **9**: 107-119

Wang, C., 1989. Studies on the Chinese sparid fishes. *Stud. Mar. Sin*., **30**: 251-259.

Wittenrich, M.L., Rhody, N.R., Turingan, R.G. and Main, K.L., 2009. Coupling osteological development of the feeding apparatus with feeding performance in common snook, *Centropomus undecimalis*, larvae: Identifying morphological constraints to feeding. *Aquaculture*, **294**: 221-227. https://doi.org/10.1016/j.aquaculture.2009.06.006

Xue, L.Y., Yang, Q.Y., Xiao, Z.K. and Li, L., 2008. Molecular characterization of myostatin in black seabream, *Acanthopagrus schlegelii*. *DNA Seq*., **19**: 217-223. https://doi.org/10.1080/10425170701517564

Yang, T. and Li, Z., 2008. A study on osteology of *Parargyrops edita* Tanaka and *Sparus latus* Houttuyn. *J. Guangdong Ocean Univ*., **28**: 1-5.

ZamboninoInfante, J.L., Cahu, C.L. and Peres, A., 1997. Partial substitution of di-and tripeptides for native proteins in seabass dietim proves *Dicentrarchus labrax* larval development. *Nutrients*, **127**: 608-614.

Zheng, P., Ma, Z., Guo, H., Zhang, D., Fu, M., Zhang, N. and Jiang, S., 2016. Osteological ontogeny and malformations in larval and juvenile golden pompano *Trachinotusn ovatus* (Linnaeus 1758). *Aquacul. Res*., **47**: 1421-1431. https://doi.org/10.1111/are.12600