Development of Skeletal Ossification in Climbing Perch (*Anabas testudineus)* from Juvenile to Adulthood

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Abstract

The climbing perch (*Anabas testudineus*) is a freshwater fish that can withstand highly unfavorable environments and stay out of the water for extended periods. Its anatomical characteristics showed terrestrial adaptation and terrestrial locomotion properties enable the use of climbing perch as an animal model. Moreover, its bone and cartilage profiles at different stages are crucial to improving the anatomical information for the osteogenesis model candidate. The current study aimed to illustrate the skeletal profiles of juvenile and adult climbing perch with the whole staining method. The samples included two adults and two juvenile climbing perch from Daerah Istimewa Yogyakarta, Indonesia. The fish were euthanized using β-hydroxyethyl phenyl ether in a lethal dose. The skeleton was examined using the Alcian blue–Alizarin red whole staining, which revealed the bones and cartilage under the stereomicroscope. The result showed that the bone is the main skeleton. Cartilage was detected in the area surrounding the orbit in the adult climbing perch and in the pterotic, pterosphenoid, prootic, and distal end of the hypural, parhypural, and basal pectoral girdle in the juvenile climbing perch. Endochondral osteogenesis was found in juvenile to adult climbing perch.

Keywords: bone, cartilage, climbing perch fish, osteogenesis, skeleton

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INTRODUCTION

The climbing perch (*Anabas testudineus*) is a savory freshwater fish consumed in Southeast Asia, including Indonesia. In Sumatra, Java, and Kalimantan, it can be found in swamps, rivers, lakes, and water ditches (Prianto *et al*., 2014). The climbing perch indicates environmental pollution as it can tolerate extremely unfavorable conditions (Zhang *et al*., 2019). Moreover, this fish has remarkable locomotion capabilities that enable them to travel long distances on land (Khan Manon *et al*., 2023; Tay *et al*., 2006). The anatomical characteristics of the climbing perch, particularly its skeletal architecture, showed terrestrial adaptation (Davenport and Matin, 1990; Hidayat *et al*., 2024).

Additionally, a comparative osteological study between the climbing perch from Thailand and that from Bangladesh showed slight differences in bone arrangement. Nevertheless, osteogenesis formation from juvenile to adult was not the primary focus of previous studies (Nabi and Ara, 2018). Information regarding the bone and cartilage profiles at different stages of the climbing perch fish is limited.

Osteogenesis is a prerequisite of cartilage and bone formation. Osteogenesis can occur from a cartilage precursor or mesenchymal condensation (Bird and Mabee, 2003). These two forms are known as endochondral and intramembranous ossification, respectively. Endochondral ossification is a process wherein the mesenchyme transforms into an intermediate cartilaginous structure resembling the future skeletal component progressively replaced by bone (Shea *et al*., 2015). Endochondral ossification occurs in all vertebrate taxa and is initiated by chondrocyte differentiation, extracellular cartilage matrix mineralization, and intermediate vascularization (Clarke, 2008; Hidayatulloh *et al*., 2021). Ultimately, the cartilage model has fully ossified, and the cartilage undergoes disintegration and resorption establishing an avascular articular cartilage (Blumer, 2021). Intramembranous ossification occurs directly, where mesenchymal cells differentiate into bone, forming flat bones including the clavicle and most cranial bones (Clarke, 2008; Sabrina *et al*., 2023; Breeland *et al*., 2023). Fish skeletons are composed of bony fish or cartilaginous fish. Fishbone formation is influenced by genetic, developmental, and environmental factors that differ between species (Claeson and Dean, 2011). Significant discoveries regarding fishbone formation have been reported. Intermuscular bones are thin bones from tendons embedded in muscle by intramembranous ossification in teleosts and are crucial for fish to swim (Li *et al*., 2022). A study on young Atlantic salmon revealed that bone formation and mineralization are independent processes (Witten *et al*., 2016). Research on the developmental timing and origin of skeletal elements in the axial skeleton of zebrafish enabled an understanding of the growth and formation of fish bones (Bird and Mabee, 2003; Syahbirin *et al*., 2024).

The present study aimed to illustrate anatomical ossification in the skeleton of the climbing perch from juvenile to adult. Bone and cartilage profiles at the juvenile and adult stages demonstrate the anatomical information of the osteogenesis model candidate. The bone formation of fish shows the mechanisms underlying the formation and skeleton mineralization. The climbing perch may be used as an animal model owing to its terrestrial locomotion properties.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia with certificate No.68/EC-FKH/INT./2023.

Study Period and Location

The study was conducted in the Daerah Istimewa Yogyakarta, Indonesia. The samples were analyzed at the Animal Systematics Laboratory, Faculty of Biology, and the Laboratory of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta.

Animal Model

The climbing perch was obtained from Progo River in the Special Region of Yogyakarta, Java, Indonesia. Juveniles had a body length of 4.19 ± 0.39 cm and weighed 2.92 ± 0.87 g (Hidayat *et al*., 2016). Two adults (weight= 14.05 g; body length= 9.7 cm and weight= 15.44 g; body length= 9.9 cm) and two juveniles (weight= 3.27 g; length= 5.9 cm and weight= 3.46 g; length= 6.2 cm) climbing perch were used as the animal models. The animals were anesthetized using β-hydroxyethyl phenyl ether (Koi Anesthesia, Keko Konishi, Japan) at a lethal dose of 0.5 mL/liter of water. An incision was performed in the ventral abdomen of the fish and fixed in 95% ethanol within 3 days for further examination.

Alizarin Red–Alcian Blue Staining

In previous study, the whole skeleton staining was modified by Bensimon-Brito *et al*., (2016). The visceral organs and scales of fish were removed and refixed in 95% ethanol for 3 days. The samples were embedded in a mixture solution of 0.3% Alcian blue (8GX. BDH, Cat. No.74240) in 70% ethanol (1 part) + 0.1% Alizarin red (Sigma Aldrich, Cat. No.A5533) in 95% ethanol (1 part) + glacial acetic acid (1 part) + 70% ethanol (17 parts) for 5 days at 37^{o}C. Then, the samples were washed using distilled water and soaked in 1% KOH solution for 5 days until the skin and muscles were seen transparently, the bone stained red, and the cartilage stained blue. Afterward, the skeletons were embedded in serial mixtures (3:1, 2:2, and 1:3, respectively) of 1% KOH and glycerol, and then fixed in a glycerol solution. The staining results were observed under a stereomicroscope (Olympus SZ2 -ILST, Japan).

Statistical Analysis

Data were analyzed descriptively based on the distribution of the Alizarin-stained bone and Alcian blue-stained cartilage.

RESULTS AND DISCUSSION

Bone and cartilage constitute the skeleton of the climbing perch teleost. The bones and cartilage were distributed in three main areas i.e., cranium, vertebrae, and fins. The bones were stained red with Alizarin red, while the cartilage was colored blue by the Alcian blue.

Cranium

In general, the ventromedial part of the cranium is composed of the os vomer anteriorly and os parasphenoid posteriorly. A pair of ossa pterosphenoids were observed at the lateral part of the os parasphenoid, and the most lateral was the os frontalis. At the caudal part of the os pterosphenoid, the ossa prootic, sphenotic, and pterotic were observed. Juvenile fish have cartilage in the lateral ossa prootic, which disappears in adulthood. The medial part of the ossa pterosphenoid consists of cartilage in juvenile and adult fish (Figure 1). In the posterior part of the juvenile and adult cranium, ossa pterotic were distributed laterally, as well as ossa epiotic caudally and ossa opisthotic medially (Figure 2). Moreover, Figure 2 shows ossa basioccipital and exoccipital at the posterior region of the os parasphenoid. Cartilage was noted between the ossa opisthotic and os parasphenoid of juveniles, whereas no cartilage was found in adult fish.

In the dorsal aspect of the cranium of the juvenile and adult fish (rostral to caudal), os prefrontal, os frontal, and otic regions were observed. Ossa sphenotic and pterotic formed in the otic region. The occipital part included the os supraoccipital and exoccipital, whereas the medial part of the caudal aspect consisted of the parietal bone. In the lateral view of the anterior cranium, the ossa orbital was visible. The suborbital area (SO) included the ossa lacrimal (SO1), jugal (SO2), and postorbital (SO3). Cartilage was visible between SO2 and SO3 (Figure 3). The ossa supraoccipital and exoccipital were observed at the roof of the occipital region from the caudal aspect of the cranium. Ventral to those bones, the ossa opisthotic and pterotic were noted in the lateral part. Foramen magnum was identified at the dorsal part of the os parasphenoid (Figure 4). The cartilage was not visible from the caudal aspect of the cranium in the juvenile and adult fish.

Vertebrae

Observation of the bony vertebrae from rostral to caudal consisted of os atlas (1 segment), os axis (1 segment), thoracic vertebrae (7 segments), precaudal vertebrae (1 segment), caudal vertebrae (15 segments), and caudal complex vertebrae. The bony vertebrae in fish were entirely made of bone, and the cartilage was not visible in juvenile or adult fish (Figure 5).

Fins

The pectoral fins comprised several fin rays embedded in fibrous pads that separated them from the underlying radials. The pectoral girdle consisted of the ossa radial, scapula, and coracoid. In juvenile climbing perch, cartilage was visible at the junction of the scapula and radial and coracoid bone, whereas in adult fish, it was fully ossified (Figure 6). Examination showed that the dorsal fin was composed of bone, and no cartilage was visible. The dorsal fin comprises the anterior dorsal fin (spine) 1–18 and the posterior dorsal fin (soft rays) 1–9 (Figure 7). Further, caudal fins were composed of several fin rays connected to the caudal skeleton (epurals, par hypural, hypural, and hemal arch) (Figure 8). In juvenile fish, the hypural and parhypural posterior end was cartilaginous. However, in adult fish, no cartilage was found.

This study was an initial observation of the skeleton features of the climbing perch by staining the cartilage using Alcian blue and the bone with Alizarin red. Known but not specified in a previous study, the skeleton structure of the climbing perch (Nabi and Ara, 2018), we illustrated the cartilage and bone distribution compared to the juvenile and adult. A limitation was noted in the age data recording of the fish; hence, the stages were determined by their size (body weight and length) according to a previous study (Hidayat *et al*., 2016). The endoskeleton in vertebrates, including fish, comprises bony fish and cartilaginous fish.

The current study found that the cartilage was distributed in some parts; thus, we believe that these structures contain hyaline cartilage, proteoglycans, and collagens. Hyaline cartilage develops first during embryonic development and

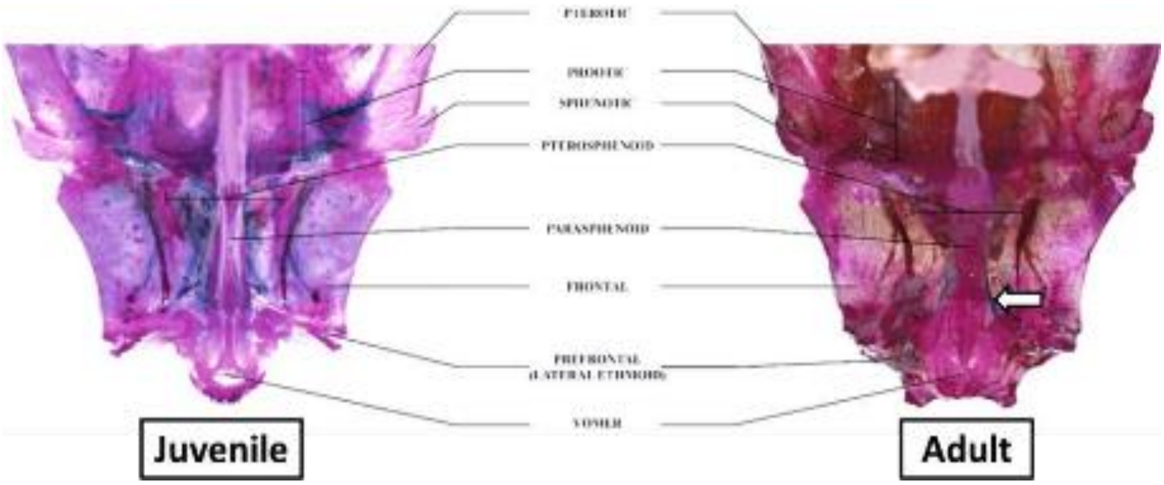

Figure 1. Ventral view of the anterior cranium area of the juvenile compared to adult climbing perch. Juveniles showed more blue-stained cartilage than adults in the lateral ossa prootic and medial ossa pterosphenoid. In adults, cartilage persists in the medial ossa pterosphenoid (arrow).

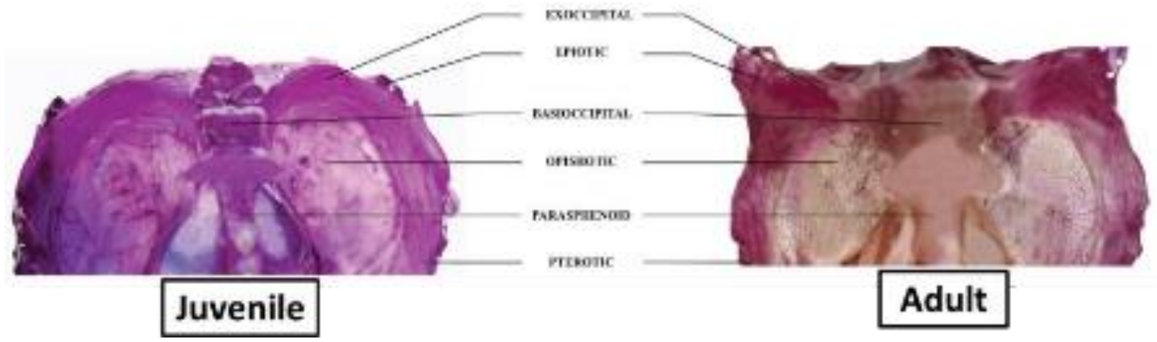

Figure 2. Ventral view of the posterior cranium area of the juvenile compared to adult climbing perch fish. The blue-stained cartilage in juveniles has fully ossified in adults.

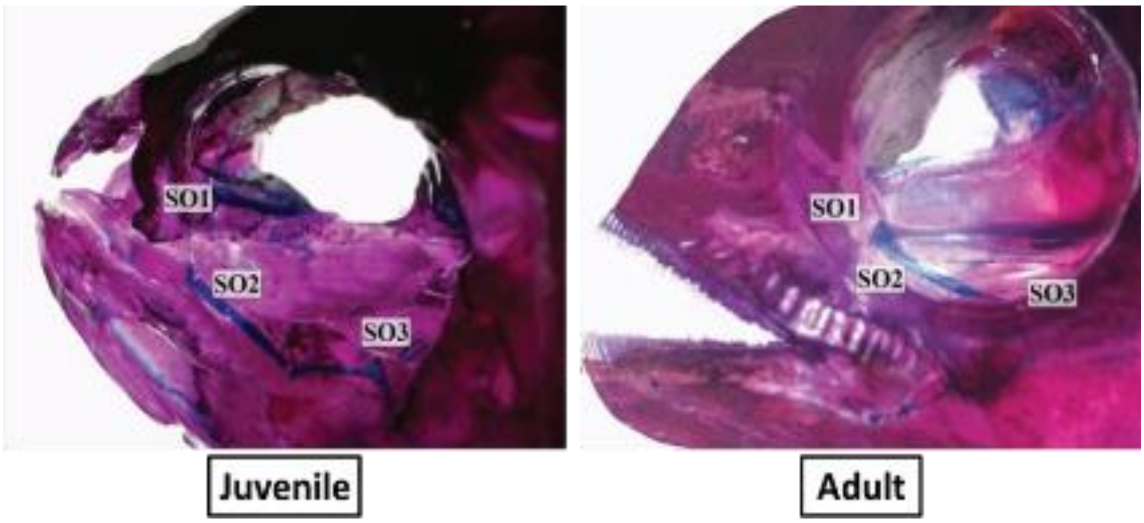

Figure 3. Lateral view of the anterior cranium area of the juvenile and adult climbing perch. The suborbital (SO), area: pterorbitals/lacrimals (SO1), jugal (SO2), and postorbital (SO3).

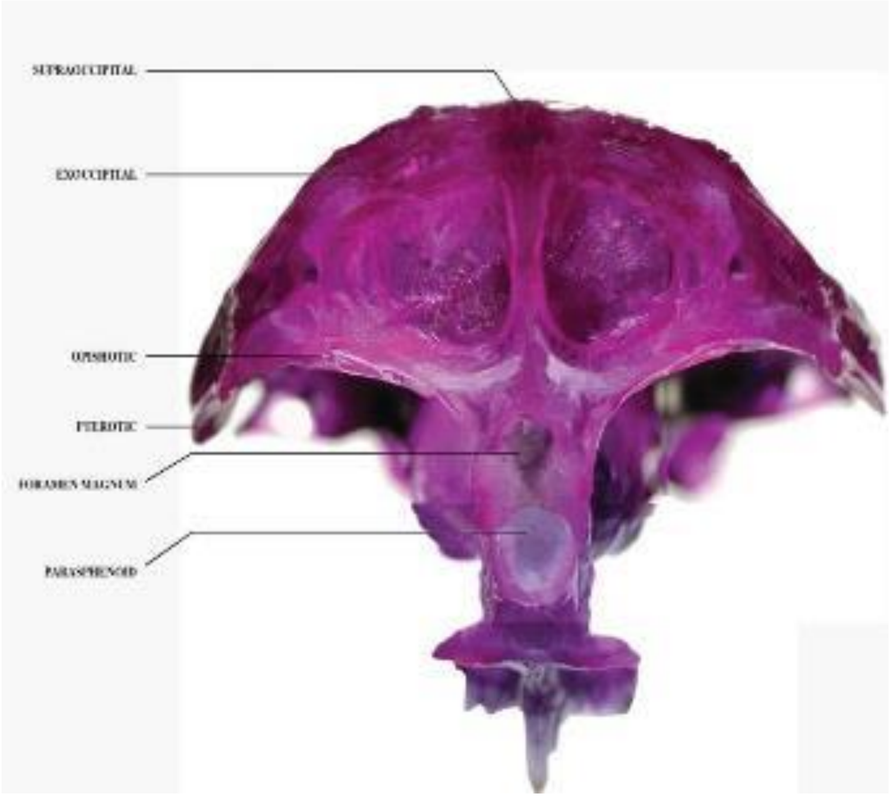

Figure 4. Caudal aspect of the cranium of the juvenile climbing perch. All bones were stained using Alizarin red.

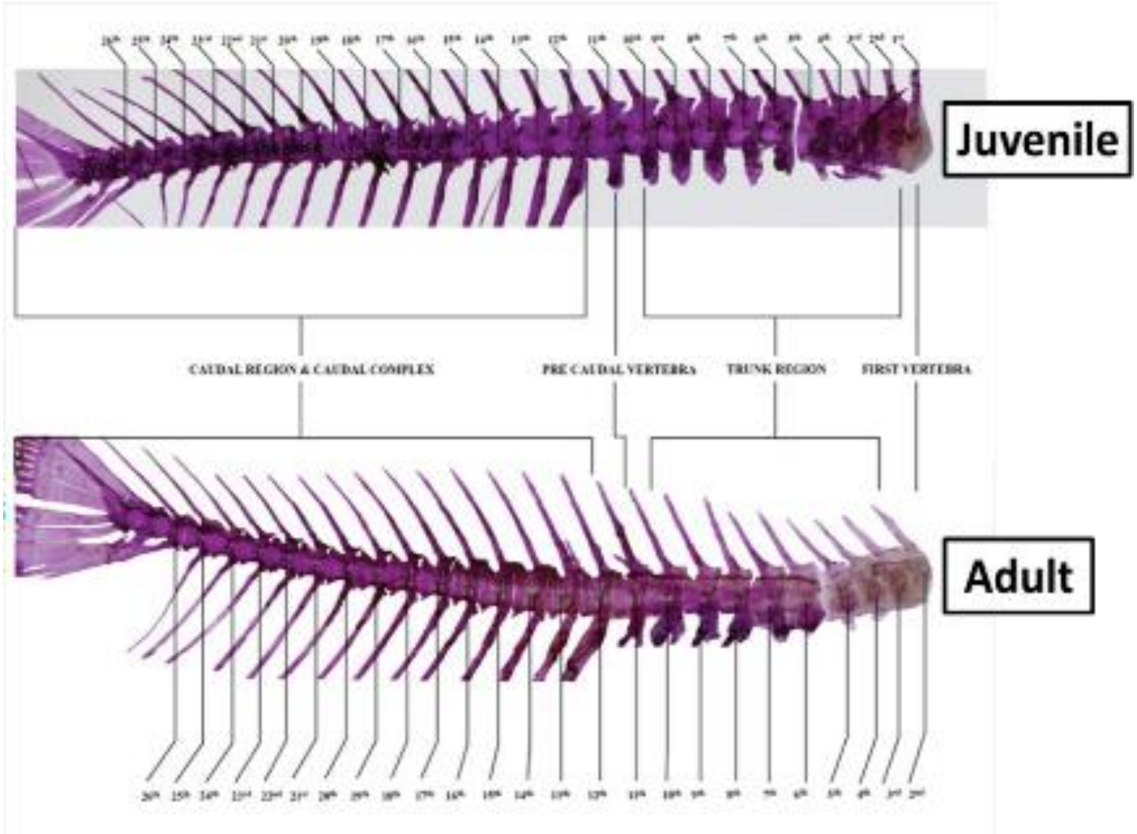

Figure 5. Alizarin red-stained whole bone vertebrae of juvenile climbing perch. The cartilage is not detected. From rostral to caudal, it consists of the atlas, axis, thoracic vertebrae, precaudal vertebrae, caudal vertebrae, and caudal complex.

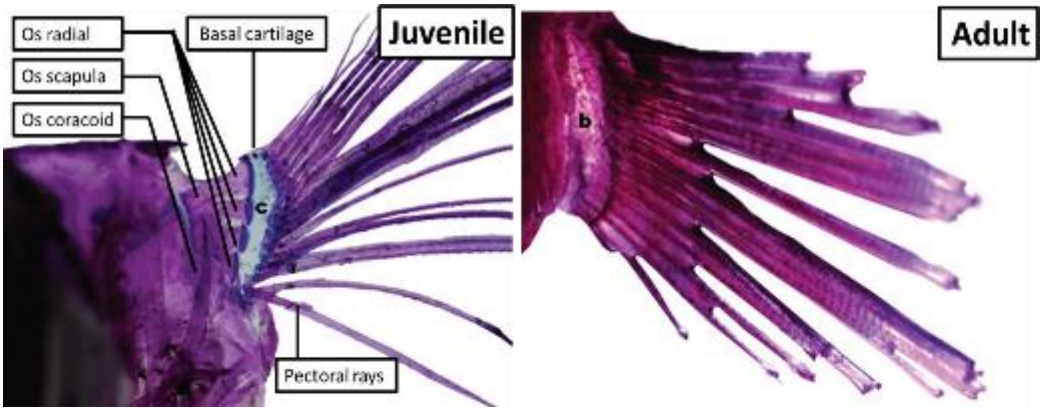

Figure 6. Pectoral fins of juvenile and adult climbing perch. In adults, Alcian blue-stained (c) cartilage turned into (b) bone.

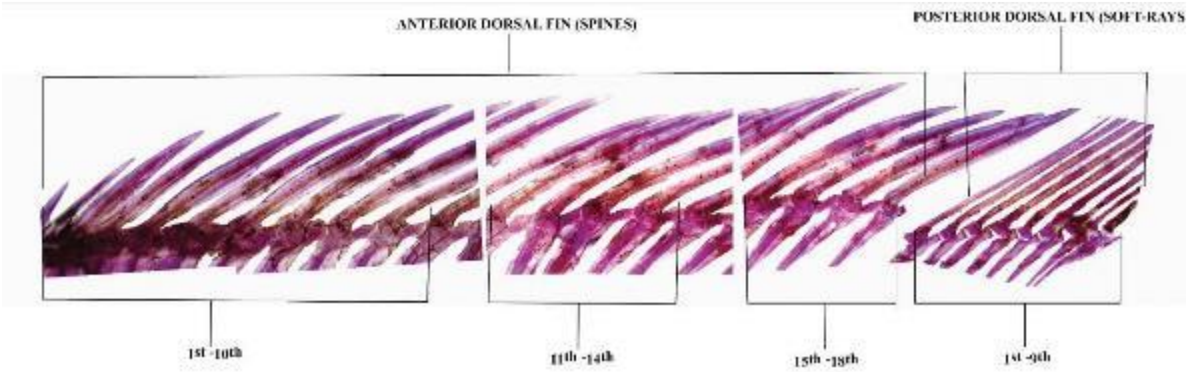

Figure 7. The dorsal fin of the adult climbing perch is composed of anterior and posterior dorsal fins.

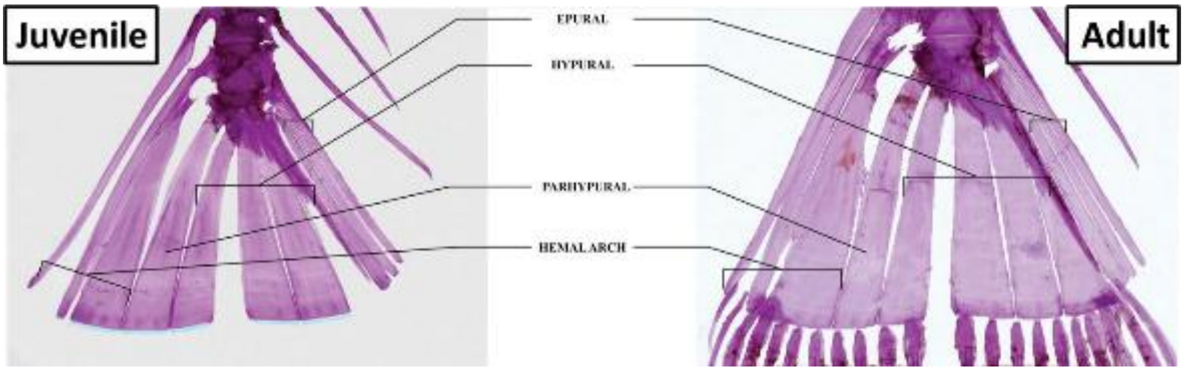

Figure 8. Caudal fins in juvenile and adult climbing perch. The cartilage is visible at the distal end of the hypural and parhypural of the juvenile fish.

serves as a template for bone formation (Benjamin, 1990). Fish cartilage is mainly comprised of proteoglycans and collagens, which are essential to the early phases of skeletal development (Li *et al*., 2021). The climbing perch's skeleton best develops in adulthood as cartilages are observable in some parts of the cranium and fins in the juvenile stage. Unlike the cartilaginous fish that retained the cartilage, they became utterly bones in adulthood. This study confirmed that the climbing perch is a bony-type fish (teleost). Approximately 41% of all bony fish belong to the Perciformes order, home to the climbing perch teleost, a member of the Anabantidae family (Khatun *et al*., 2019).

Comparing the distribution of the cartilage and bones from juvenile to adult fish indicates an osteogenesis process similar to that of mammals, as revealed in a study on zebrafish (Bergen *et al*., 2019). However, some persistent cartilage showed slow growth. The bone growth of the climbing perch is associated with diet, nutritional value, and growth performance (Ahammad *et al*., 2021; Ismarica *et al*., 2020; Kader *et al*., 2011).

Studies on muscle cellularity, growth performance, and growth-related gene expression showed that juvenile climbing perch exhibits a slow growth pattern during the culture period (Ahammad *et al*., 2021). In higher vertebrates, osteogenesis occurs through endochondral and intramembranous ossifications (Blumer, 2021; Breeland and Menezes, 2023). Distribution of the cartilage in the lateral ossa prootic and between the ossa opisthotic and os parasphenoid cranium, pectoral girdle, and caudal fin border of the juvenile climbing perch indicates that osteogenesis occurs through endochondral ossification in those areas. Endochondral ossification is the process by which the bone replaces the cartilage in the growing skeleton (Mackie *et al*., 2008). Although most bones grow via endochondral ossification, osteogenesis involves several stages of development, including cell differentiation, proliferation, maturation of the matrix, and mineralization (Rutkovskiy *et al*., 2016). Histological features and factors underlying the process of osteogenesis in climbing perch remain to be clarified. Intramembranous ossification, wherein mesenchymal cells differentiate into bone (Setiawati and Rahardjo, 2019; Breeland and Menezes, 2023), possibly occurs in the bones located at the dorsal cranium, considering our findings showed no cartilage in those areas. Despite this, cranial bones are separated by connective tissue with a distinctive architecture of osteogenic cells and collagen fibrils (Topczewska *et al*., 2016), which may be unstained with Alcian blue.

Briefly, most of the bones in the skeletons of climbing perch found in Bangladesh and Thailand were structurally similar despite some variances (Nabi and Ara, 2018) as the current study noted an additional vertebral segment in the caudal region. Little genetic diversity is observed within populations. Given that the species exhibits intraspecific phenotypic differences connected to particular habitats, the limited genetic variation may be related to habitat adaptations (Khan Manon *et al*., 2023). The tail and pectoral fins of the climbing perch are beneficial for their mobility, enabling crawling or wriggling on land and possibly going several hundred meters (Tay *et al*., 2006). These data demonstrate that the climbing perch can effectively explore aquatic and terrestrial habitats because of its unique terrestrial locomotion properties (Davenport and Matin, 1990). The characteristics found in the current study indicate that the climbing perch is a potential model for osteogenesis in higher vertebrates.

The appendicular skeleton in fish consists of the bones of the paired appendages, including their girdles, and the unpaired appendages. Small proximal components such as pterygiophores and bone rays provide support for all fins (Claeson and Dean, 2011). The appendicular skeleton of zebrafish is not homologous to that of humans. The osteogenesis of the exoskeletal ray’s fin was intramembranous and was endochondral in the hypural (Pabic *et al*., 2022).

In fish, the skull is a hard structure to which interdependent parts such as mandibles, gill arches, opercula, and branchiostegal rays are attached, several of which participate in breathing and feeding. The axial skeleton of fish consists of the skull and spine. In the axial skeleton, there is a jugal bone which is part of the cheek area and plays a role in protecting the eye area and related soft tissues and attaching muscles involved in jaw movement (Claeson and Dean, 2011). However, we have amount limitation on the animal due to the samples collected from nature.

CONCLUSION

Endochondral osteogenesis is observed in juvenile to adult climbing perch that demonstrate slow bone growth. Further study of a more comprehensive skeleton profile, including histological features is required.

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AUTHORS' CONTRIBUTIONS

TWP: Conceptualization, Supervision, Resources, Project Administration, Funding Acquisition. WDW: Investigation, Visualization, Formal Analysis, Writing-Original Draft. DLK: Methodology, Software, and Validation. TWP, WDW, and DLK: Writing Review and Editing. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Ahammad, A. K. S., Asaduzzaman, M., Uddin Ahmed, M. B., Akter, S., Islam, M. S., Haque, M. M., Ceylan, H., & Wong, L. L. (2021). Muscle cellularity, growth performance and growth-related gene expression of juvenile climbing perch *Anabas testudineus* in response to different eggs incubation temperature. *Journal of Thermal Biology*, 96, 1–8.

Benjamin, M. (1990). The cranial cartilages of teleosts and their classification. *Journal of Anatomy*, 169, 153–172.

Bensimon-Brito, A., Cardeira, J., Dionísio, G., Huysseune, A., Cancela, M. L., & Witten, P. E. (2016). Revisiting in vivo staining with alizarin red S - A valuable approach to analyse zebrafish skeletal mineralization during development and regeneration. *BMC Developmental Biology*, 16(1), 1–9.

Bergen, D. J. M., Kague, E., & Hammond, C. L. (2019). Zebrafish as an emerging model for osteoporosis: A primary testing platform for screening new osteo-active compounds. *Frontiers in Endocrinology*, 10(6), 1–20.

Bird, N. C., & Mabee, P. M. (2003). Developmental Morphology of the Axial Skeleton of the Zebrafish, *Danio rerio* (Ostariophysi: Cyprinidae). *Developmental Dynamics*, 228(3), 337–357.

Blumer, M. J. F. (2021). Bone tissue and histological and molecular events during development of the long bones. *Annals of Anatomy*, 235, 1–11.

Breeland, G., Sinkler, M. A., & Menezes, R. G. (2023). Embryology, Bone Ossification. In *StatPearls*. http://www.ncbi.nlm.nih.gov/pubmed/30969540

Claeson, K. M., & Dean, M. N. (2011). The skeleton | Cartilaginous Fish Skeletal Anatomy. In *Encyclopedia of Fish Physiology*, 1, 419–427.

Clarke, B. (2008). Normal bone anatomy and physiology. *Clinical Journal of the American Society of Nephrology : CJASN*, 3(Suppl 3), 131–139.

Davenport, J., & Matin, A. K. M. A. (1990). Terrestrial locomotion in the climbing perch, *Anabas testudineus* (Bloch) (Anabantidea, Pisces). *Journal of Fish Biology*, 37(1), 175–184.

Hidayat, R., Carman, O., & Alimuddin, A. (2016). Sexual dimorphism related to growth in climbing perch *Anabas testudineus*. *Jurnal Akuakultur Indonesia*, 15(1), 8–14.

Hidayat, R., Carman, O., & Alimuddin, A. (2024). Early Sex Differentiation of Climbing Perch (*Anabas testudineus* Bloch.): A Pathway to Feminization. *Jurnal Medik Veteriner*, 7(1), 143–154.

Hidayatulloh, D. R., Dhamayanti, Y., & Purnama, M. T. E. (2021). Species determination based on head scutes, carapace, and plastron of turtle hatchlings at Boom Beach, Banyuwangi. *IOP Conference Series: Earth and Environmental Science*, 718(1), 012047.

Ismarica, I., Setiawati, M., Jusadi, D., & Suprayudi, M. A. (2020). Bone formation and growth of climbing perch *Anabas testudinieus* larvae fed with Zn enriched Artemia nauplii. *Jurnal Akuakultur Indonesia*, 19(2), 153–159.

Kader, M. A., Bulbul, M., Ahmed, G. U., Hossain, M. S., Hossain, M. A., & Koshio, S. (2011). Effects of animal proteins in practical diets on growth and economic performance of climbing perch, *Anabas testudineus* (Bloch). *Journal of Applied Aquaculture*, 23(2), 166–176.

Khan Manon, M. R., Alam, A., Ullah, M. R., Hossen, M. B., Sufian, M. A., Hossain, M. A., Iqbal, M. M., & Rahman, M. A. (2023). Intraspecific phenotypic differences in climbing perch *Anabas testudineus* (Bloch, 1792) populations may be linked to habitat adaptations. *Heliyon*, 9(7).

Khatun, D., Hossain, M. Y., Rahman, M. A., Islam, M. A., Rahman, O., Azad, M. A. K., Sarmin, M. S., Parvin, M. F., Ul Haque, A. T., Mawa, Z., & Hossain, M. A. (2019). Life-History Traits of the Climbing perch *Anabas testudineus* (Bloch, 1792) in a Wetland Ecosystem. *Jordan Journal of Biological Sciences*, 12(2), 175–182.

Li, B., Zhang, Y. W., Liu, X., Ma, L., & Yang, J. X. (2021). Molecular mechanisms of intermuscular bone development in fish: A review. *Zoological Research*, 32(3), 362–376.

Li, W., Ura, K., & Takagi, Y. (2022). Industrial application of fish cartilaginous tissues. *Current Research in Food Science*, 5(April), 698–709.

Mackie, E. J., Ahmed, Y. A., Tatarczuch, L., Chen, K. S., & Mirams, M. (2008). Endochondral ossification: How cartilage is converted into bone in the developing skeleton. *International Journal of Biochemistry and Cell Biology*, 40(1), 46–62.

Nabi, M. R., & Ara, I. (2018). International Journal of Fisheries and Aquatic Studies 2018; 6(4): 484–491 Osteological comparison between local and Thai climbing perch in terms of neurocranium, vertebral column and accessory respiratory organ. *Ijfas*, 6(4), 484–491.

Prianto, E., Kamal, M. M., Muchsin, I., & Kartamihardja, E. S. (2014). Biologi Reproduksi Ikan Betok (*Anabas testudineus*) di Paparan Banjiran Lubuk Lampam, Kabupaten Ogan Komering Ilir. *BAWAL Widya Riset Perikanan Tangkap*, 6(3), 137–146.

Rutkovskiy, A., Stensløkken, K. O., & Vaage, I. J. (2016). Osteoblast Differentiation at a Glance. *Medical Science Monitor Basic Research*, 22, 95–106.

Sabrina, A. N., Mukti, A. T., Suciyono, Kenconojati, H., Ulkhaq, M. F., Fasya, A. H., Lamadi, A., Imlani, A., & Mariah, S. R. (2023). Color brightness and growth levels of goldfish (*Carassius auratus*) reared with different light spectrums. *Jurnal Medik Veteriner*, 6(2), 250–255.

Setiawati, R., & Rahardjo, P. (2019). Bone Development and Growth. *Osteogenesis and Bone Regeneration*, 1–20.

Shea, C. A., Rolfe, R. A., & Murphy, P. (2015). The importance of foetal movement for co-ordinated cartilage and bone development in utero: clinical consequences and potential for therapy. *Bone & joint research*, 4(7), 105–116.

Syahbirin, G., Aditianingrum, K. A., & Mohamad, K. (2024). Acute Toxicity of Ethanol Extract of *Curcuma zedoaria* Rosc (Zingiberaceae) Rhizomes on Brine Shrimp Larvae and Zebrafish Embryos. *Jurnal Medik Veteriner*, 7(1), 7–18.

Tay, Y. L., Loong, A. M., Hiong, K. C., Lee, S. J., Tng, Y. Y. M., Wee, N. L. J., Lee, S. M. L., Wong, W. P., Chew, S. F., Wilson, J. M., & Ip, Y. K. (2006). Active ammonia transport and excretory nitrogen metabolism in the climbing perch, *Anabas testudineus*, during 4 days of emersion or 10 minutes of forced exercise on land. *Journal of Experimental Biology*, 209(22), 4475–4489.

Topczewska, J. M., Shoela, R. A., Tomaszewski, J. P., Mirmira, R. B., & Gosain, A. K. (2016). The morphogenesis of cranial sutures in zebrafish. *PLoS ONE*, 11(11), 1–23.

Witten, P. E., Owen, M. A. G., Fontanillas, R., Soenens, M., Mcgurk, C., & Obach, A. (2016). A primary phosphorus-deficient skeletal phenotype in juvenile Atlantic salmon Salmo salar: The uncoupling of bone formation and mineralization. *Journal of Fish Biology*, 88(2), 690–708.

Zhang, W., Xie, H. Q., Li, Y., Jin, T., Li, J., Xu, L., Zhou, Z., Zhang, S., Ma, D., Hahn, M. E., & Zhao, B. (2019). Transcriptomic analysis of *Anabas testudineus* and its defensive mechanisms in response to persistent organic pollutants exposure. *Science of the Total Environment*, 669(86), 621–630.

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