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Skeletal Deformities in Cultured Juvenile African Catfish *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

Skeletal deformities in cultured fish are known worldwide and it had affected the survival, growth and appearance of the fish which contribute to production loss. *Clarias gariepinus* is known as number one farmed fish in Malaysia. *C. gariepinus* also effected in these deformities especially in cultured species. The objectives of this study are to identify the skeletal deformities that occur in juvenile stages and to compare the bone structure between the normal and deformed fish. A total of 50 juveniles' fish were collected from Aquaculture Extension and Community Centre Machang, Kelantan. The juvenile was measured before undergo staining process. The fish were starved for one day then fixed in 70% ethanol for two weeks. The fish were then stained with alizarin red S for cartilage and Alcian blue for bone. The specimens were photographed in order to observe the deformities. The data showed only 13.0% juveniles have skeletal deformities, 10.9% with lordosis and 2.2% scoliosis. The cause of the deformities may be due to the heterogenous growth but other factors might also contribute to the problem. As the conclusion, the skeletal deformities observed in juvenile *C. gariepinus* were detected with the whole-mount staining method ranged from size 7.3 and 26.0 cm in total length. This study shows there is heterogenous growth rearing at low percentage even from skilled farm. Further study should concentrate on hidden factors that affected the deformity rate and mineralization of the juvenile fish.

Keywords: Bone staining, *Clarias gariepinus*, Deformities, Juvenile fish, Skeletal

INTRODUCTION

Deformity is a major problem in fish industry because it can affect both welfare and production of the fish. Due to this problem, the ability of fish with deformities to fight for food, their growth performance, and survival rate reduce compared to normal fish and the market price of the fish decrease considering its unappealing appearance which later become a major problem for the fish industry (Boursiaki et al, 2019; Fernández et al 2021). Deformity is vital to study to understand the root cause of the problem and to maximize the seed production especially for small scale farmers.

One of the most crucial pathological effects in fishes is skeletal deformities. It affects several areas of the skeleton including the feeding apparatus and suspensorium, fins, vertebrae, neurocranium, ribs, and vertebral column. Lordosis and kyphosis (dorso-ventral column) and scoliosis (lateral curvature) are the most frequent deformities found in fish cited in (Fatnassi et al., 2017).

African catfish (*Clarias gariepinus*) is an African native freshwater catfish. They are hardy, easily to produce, and matures quickly (Awe, 2017). It is one of the most cultured species and grown rapidly to become the major produced finfish in Malaysia which overrun the red tilapia (Dauda et al., 2018). *C. gariepinus* was introduced in Malaysia's aquaculture since 1980's from Thailand, and since then it grown moderately but the production of the *C. gariepinus* experienced up and down and it gave big impact towards the aquaculture industry in Malaysia (Dauda et al., 2018).

C. gariepinus is known as 'Ikan keli' in Malaysia. They have been a popular choice among farmers in culturing fish because of its rapid growth. *C. gariepinus* accounts for 10% of total aquaculture production in Malaysia (Dauda et al., 2018). Farmers can harvest their fish in three months in which they easy to maintain and do not require high cost. Several studies like temperature manipulation, gynogenetic diploid, breeding, and rearing have been carried out on *C. gariepinus* for aquaculture improvement (Mwanja et al., 2015). Even though the effort for aquaculture improvement have achieved, the seed production techniques yet to be established and seed supply is inconsistent due to low survival rate. Skilled farmer also not exempted from having fish with deformity even in low rate.

C. gariepinus is prone to vast variety of diseases which include fungi, bacteria and diverse parasites. Abnormalities in fish differs tremendously not just in between different fishery, different lots inside the similar hatchery and even in the same group of eggs. The percentage of the abnormalities whether standard or severe also have a big gap. Complex combination of multiple bone disorder and spinal deformity is entitled deformities such as lordosis, kyphosis, scoliosis and also vertebrae fusion (Berillis, 2015). Numerous skeletal deformities in reared fish have lack of systematic classification. Various terminologies without lengthy explanation of the affected bones are used. The objectives in this study are to identify the skeletal deformities in juvenile *C. gariepinus* and to compare the bone structures of *C. gariepinus* between normal and deform fish.

MATERIAL AND METHODS

Preparation of Fish Specimen

A total of 50 individuals of juvenile *C. gariepinus* collected from Aquaculture Extension and Community Centre Machang, Kelantan. The fish were kept in a water tank and starved for one day to empty their alimentary canal. The fish then euthanized in cold water for about 30 minutes (Sakata-Haga et al., 2018). Measurement is very necessary as a record. The measurement taken including total length (TL, cm), standard length (SL, cm), body height (cm) and body weight (g) using ruler and weight with weighing scale.

Fixation and staining process

The fish specimens were undergone fixation, cartilage and bone staining, clearing process and preservation. The juvenile fish were fixed in 70% ethanol for two weeks (Schnell et al., 2016). Staining process follow method of Liao et al. (2020). The specimen stained for accentuate the visualization of cartilage and bone to differentiate one fish to another and also to determine the specific structures. Mixtures of 10 mg Alcian blue stain, 80 ml 95% ethanol, and 20 ml glacial acetic acid were prepared for the cartilage staining in a glass jar. The specimens were placed in the mixture for about 24 to 48 hours. The specimen need to be examined twice per day to make sure that the Alcian blue solution stained specimen well and the specimen should not be left for too long in the mixtures. The specimens were then rehydrated into 70%, 35% and 17.5% of ethanol, and 1% Potassium Hydroxide (KOH), respectively for a day. Dehydration is to de-stain and clear the specimen before the bone staining. KOH solution needs to be change immediately once become turbid.

Clearing and preservation

The specimens were stained with 0.001% Alizarin red S (1% Alizarin red in 1% KOH) for about a day. Then, immersed in 1% KOH solution three changes every two hours. The specimens are then cleared in a series of 20%, 50%, 80% glycerol in 1% KOH, respectively, with 24 hour per change. Stained juveniles were preserved in 100% glycerol (Liao et al., 2020).

Skeletal Structure Analysis

The specimens were photographed and macroscopic identification of skeletal structure was determined by compare between deformed and normal fish. The data were collected and examined for t-test analysis. Results are expressed as means ± standard deviations.

RESULTS AND DISCUSSION

The notable features of normal and deformed *C. gariepinus* juvenile obtained in this study are presented in Figure 1. Size of juvenile taken from the farm were ranged from 7.3 cm to 26.0 cm in total length (14.2±6.6 cm), 12.1±5.6 cm in SL, 2.9±2.1 cm in body height and 7.7±5.0 g in body weight. The differences shown that the juveniles were put in heterogenous growth. This can cause social hierarchy and competition for food. Juveniles pirarucu during weaning and juvenile reared in cages at various stocking densities shown aggressiveness among individuals in heterogenous growth cited in (Lima, 2020). This also can cause deformities in fish as they need to compete to get enough nutrient to grow. Spinal deformities had shown to delayed growth of Far Eastern catfish (Yang et al., 2015).

By using whole mount staining procedures, deformed fish found were only 13% while normal fish were 87%. These results showed that only small amounts of juvenile fish have deformities. The study of skeletal development is needed as fish are amongst the most evolved and diversified taxonomic group, with phenotypes ranging from simple to complex. The quality of fish can be determined by the development of skeletal during juvenile stages. The occurrence of deformities in farmed fish can come from many aspects, such as diet, handling, and environment. Farmed and wild fish have the occurrence of deformities whether on their fins, scales and even in skeleton. These deformities occur during the development stages which are in larvae and juvenile. This happened due to the nutritional factors, genetic, physiological, and environmental (Filho et al., 2018).

It had been reported that deformity in *C. gariepinus* comes in various forms. Deformity in pectoral fin, mouth, big head, and lack of tail cited in (Awe, 2017). Skeletal deformities disrupt the development process of the skeletal resulting in the morphological deformities and the growth of the fish retarded and almost customers have little to no interest in buying the deform fish (Alarape et al., 2015; Berillis, 2015; Awe, 2017). Head and body are the common deformities found in *C. gariepinus* (Alarape et al., 2015) and the deformities noticeable during larval stage and juvenile (Alarape et al., 2015; Awe, 2017).

The skeletal deformities observed in the juvenile *C. gariepinus* were lordotic on abdominal region and caudal region (10.9%) and scoliosis (2.2%) (Figure 1). The most common skeletal deformities observed in this study was lordosis and it had been reported that the most common deformities in fish (Fatnassi et al., 2017). The causative factors could not be totally identified for skeletal deformities because of different external parameters in the hatcheries. Larval stages in the hatchery were fed with formulated moist feed and the juvenile fed with commercial fish feed. Balanced diet that enriched with essential fatty acids should be used in larval and juvenile stage to reduce nutrition deficiencies, which then can lead to deformities such as scoliosis and lordosis (Fatnassi et al., 2017).

This experiment use bone staining method because this method can give better picture of bones in small vertebrate compared to X-ray. Previous study on vertebral abnormalities may go unnoticed by the researcher when aberration diagnosed on high resolution radiography (Johnson et al., 2020). The staining for cartilage and bone were done separately for better stain. The percentage of KOH used in this method for replacement of mixed solutions in glycerol and KOH was higher compared to normal method which was 0.5%. Higher KOH concentration had caused disintegration of the tissue on vertebrate (Liao et al., 2020).

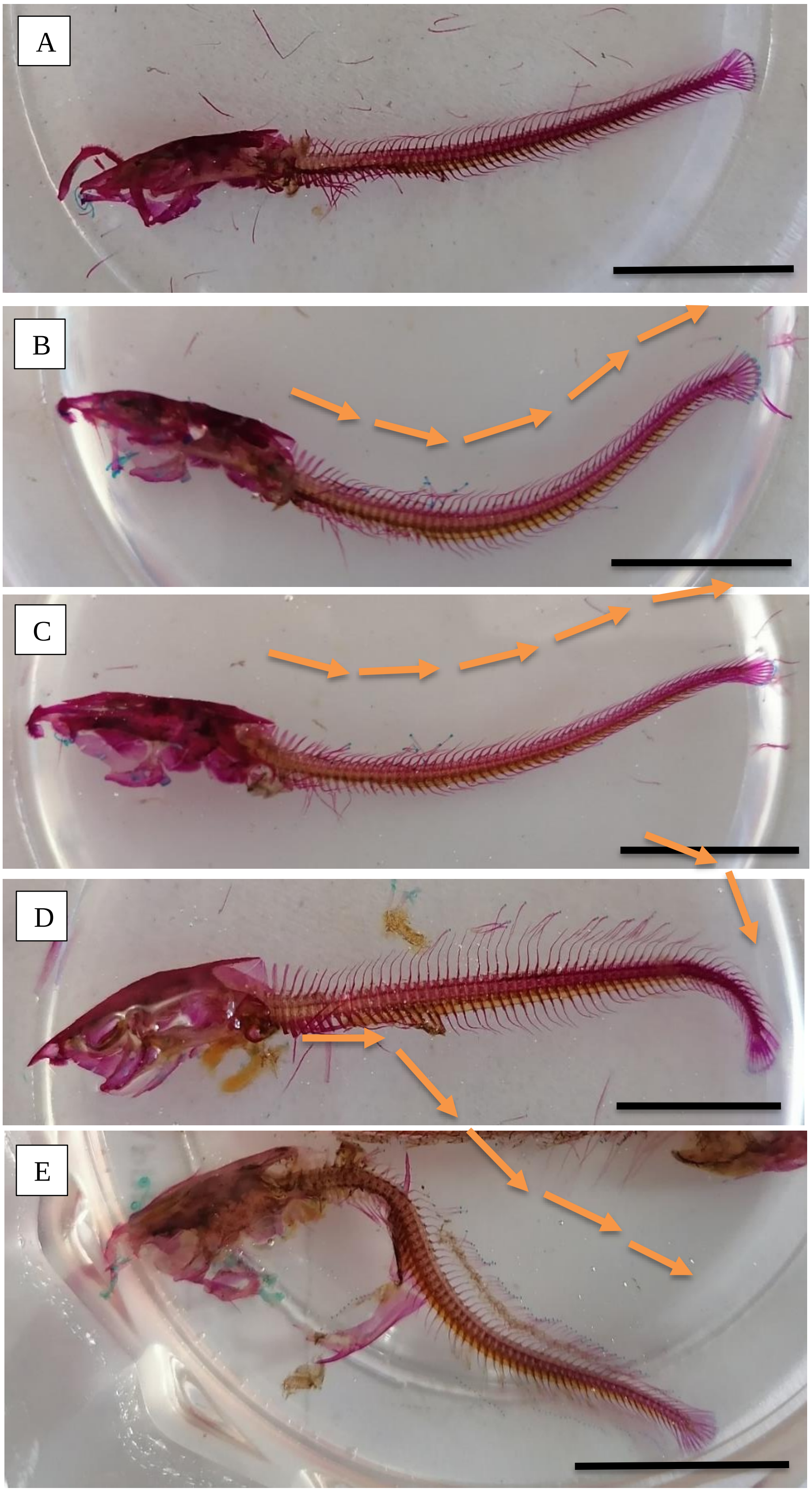

Figure 1: Stained specimen of normal and deformed juvenile. Arrow showed the deformities on fish specimen. (A) normal juvenile (B & C) lordotic in abdominal region (D) lordotic in caudal region, and (E) scoliosis. (Scale, 1cm)

CONCLUSION

In this study, skeletal deformities observed in juvenile *C. gariepinus* were detected with the whole-mount staining with ranged from 7.3 to 26.0 cm in TL, 13% of the juvenile had spinal deformities, lordosis (10.9%) and scoliosis (2.2%). This study shows there is heterogenous growth rearing at low percentage even from skilled farm. Further study should concentrate on hidden factors that affected the deformity rate and mineralization of the juvenile fish.

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