

Short Communication

Skeletal anomalies in dusky grouper *Epinephelus marginatus* larviculture

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ABSTRACT. The production of fingerlings on a large scale is one of the biggest challenges for grouper aquaculture development. Groupers may be more susceptible to skeletal anomalies due to their complex skeleton formation ontogenesis. Such anomalies are normally associated with inadequate larviculture protocols for marine fish. The present study aimed to record the occurrence of skeletal anomalies in dusky grouper *Epinephelus marginatus*. Different degrees of kyphosis, lordosis, and scoliosis were identified. After the larviculture period (60 days), 42% of the dusky grouper presented skeletal anomalies. Therefore, we have concluded that the current protocol for the dusky grouper larviculture must be re-examined, especially to determine a more appropriate water flow in the tanks.

Keywords: *Epinephelus marginatus*; larviculture; reproduction; marine finfish; mariculture; lordosis; skeletal anomalies

The sustainability of the exploitation of aquatic resources is an issue that has been considered in recent studies (Kuhnen et al. 2019). Aquaculture is an activity that has been growing in different countries, given the potential for using the waters in food production (Herrera et al. 2019). Despite its continuous growth, tropical marine finfish culture in Brazil is still incipient, as technological expertise for developing many of the species demanded by the consumer market (e.g. tuna fish and flounder) is non-existent (Sanches et al. 2014, Tosta et al. 2019). The dusky grouper, *Epinephelus marginatus* (Lowe, 1834), is of great economic importance, weighing up to 60 kg; it is very much appreciated in commercial and recreational fishing (Sousa et al. 2019). The production of fingerlings on a large scale is one of the biggest challenges for grouper aquaculture development. Marine fish larviculture presents numerous complexities ranging from appro-

appropriate feed to comply with the requirements of each species to the maintenance of environmental parameters suitable to the larval development, such as temperature, oxygen concentration, and ammonia levels (Planas & Cunha 1999). The scarcity of information about each species has resulted in inadequate larviculture protocols, which have led to a high occurrence of skeletal anomalies in marine finfish (Boglione et al. 2009). Skeletal anomalies result in shortened bodies, enlarged fins, and curved spinal columns (Russo et al. 2011). Skeletal anomalies occur spontaneously in nature (Brito et al. 2019). However, deformed specimens are rarely recorded in natural environments, possibly to their increased susceptibility to predation (Gavaia et al. 2002, Jawad 2004, Kayim et al. 2010, Bueno et al. 2015). Deformed fish in captivity are subjected to at least a year of stress, the minimum period of a fattening cycle. Therefore, assessing skeletal

anomalies is of the utmost importance due to farming profitability and individual welfare (Boglione et al. 2013).

Skeletal anomalies and their incidences are one of the most important factors affecting marine larviculture, with effects on production costs, taking into account that as many as 50-60% of hatchery juveniles may be affected (Kayim et al. 2010). Losses due to anomalies also impact on-growing farms, where malformed market-size fish must be discarded or sold at lower prices than market prices (Sanches et al. 2015). Skeletal anomalies are reported in several marine species used in aquaculture, including Atlantic salmon (*Salmo salar*), sea bass (*Dicentrarchus labrax*), and sea bream (*Sparus aurata*) (Russo et al. 2011). Skeletal malformations can be defined as kyphosis, the deviation of the spine projecting the head to one side. Lordosis is the increase in the lumbar curvature towards the abdomen and scoliosis, which is the lateral deviation of the spine in a C or S shape (Berillis 2015).

Grouper aquaculture is a promising activity. However, its sustainability depends on controlled reproductive processes and the capacity for the production of high-quality juveniles (Silva et al. 2018, Paixão et al. 2020). Species with longer larval periods, such as groupers of the genus *Epinephelus*, are more susceptible to anomalies due to the complex ontogenesis of its skeleton formation (Boglione et al. 2009, Russo et al. 2011, Sanches et al. 2015). Groupers are usually found in coastal areas and possess a high market value; as a result, many species are endangered (Pierre et al. 2008). The dusky grouper is an example of an endangered species produced commercially (Sousa et al. 2019). The present study aimed to report the occurrence of skeletal anomalies in dusky grouper fingerlings produced in captivity according to the larviculture protocol that Kerber et al. (2012) recommended.

In order to avoid the occurrence of anomalies resulting from inbreeding, non-related wild breeders were used ($\sigma = 3$ and $\varphi = 1$) (Brazilian Institute of Environment IBAMA N°164; 02027.002626/2005-09 and Ethics Committee on Animal Use of the Fisheries Institute - CEEAIP 05/2014). Reproduction was conducted under natural conditions (without any hormonal manipulation). Around 200,000 viable eggs were produced. After hatching, the larvae were stocked at a density of 4 L⁻¹, and larviculture was executed according to Kerber et al. (2012) protocols. Larviculture feeding involves the use of rotifers between days 2 to 18 (enriched with Easy Dry Selco® for 12 h) as the first food, followed by using *Artemia* sp. (nauplius) between days 12 to 22 and *Artemia* sp. (metanauplius) between days 18 to 36 (enriched with

Easy Dry Selco® for 24 h). Easy Dry Selco® composition: 27% crude protein, 28% crude oils and fats, 95 mg g⁻¹ DHA, 5 mg g⁻¹ EPA, vit. A 70,000 IU kg⁻¹, vit. D3 23,500 IU kg⁻¹, vit. C 16,500 mg kg⁻¹ and vit. E 4950 mg kg⁻¹. Weaning (phase of changing the feeding regime of live prey to the inert diet) started from 34 DAH with a commercial diet with 55% crude protein and 16% crude oils and fats. Larviculture water quality was measured twice each day. Data are reported as mean \pm standard deviation (SD): dissolved oxygen (4.5 \pm 0.6 mg L⁻¹), oxygen saturation (75.8 \pm 8.9%), oxide-reduction potential (325 \pm 38.4 μ S cm³), temperature (26.1 \pm 1.5°C), ammonia (0.03 \pm 0.02 mg L⁻¹), nitrite (0.04 \pm 0.02 mg L⁻¹), nitrate (6.30 \pm 3.41 mg L⁻¹), phosphorus (0.03 \pm 0.01 mg L⁻¹) and total alkalinity (11.81 \pm 1.31 mg L⁻¹). The values were within the range considered suitable for marine fish larviculture. At the end of the larviculture period (60 days), 700 fingerlings remained.

Grouper fingerlings were euthanized with 0.1 g L⁻¹ benzocaine and sent for radiographic image capture. The radiographs were performed in right lateral and dorsoventral projection, and a computerized radiographic system acquired the images (KODAK®) using an AGFA-Health Care CR 30x digitizer, using a radiographic technique of 45 kVp, 100 mA, and 0.025 s. Subsequently, they underwent intramuscular injection of 10% formalin solution. After 24 h, they were placed in 70% alcoholic solution. Complementing the evaluations, the animals were diaphanized by the technique of Ribeiro et al. (2011).

Forty-two percent of the sampled grouper fingerlings presented skeletal anomalies. Thirty groupers were selected and evaluated for skeletal anomalies. Dorsal, ventral and lateral deviations were identified, characterizing different degrees of kyphosis, lordosis, and scoliosis (Fig. 1). Several fish had more than one type of skeletal anomaly. The length of the fish's spinal column without anomalies was 3.6 \pm 0.2 cm; in fish with skeletal anomalies, 2.7 \pm 0.2 cm. Shortening of the vertebral column in affected fish ranged from 13.2 to 34.2%. The angles of kyphosis and lordotic deviations ranged from 65 to 154°. Scoliosis angles ranged from 61 to 135°.

Such anomalies indicate that the larvae were submitted to inappropriate conditions for their development. The causes of skeletal anomalies can be attributed to several factors. The sources of marine fish anomalies could be related to conditions during larval development, which is affected by feeding, stocking density, water temperature, and salinity (Boglione et al. 2009). Skeletal deformities such as lordosis, scoliosis, and kyphosis can occur in farmed fish due to nutritional deficiency (Oliveira et al. 2020). In the present case, the

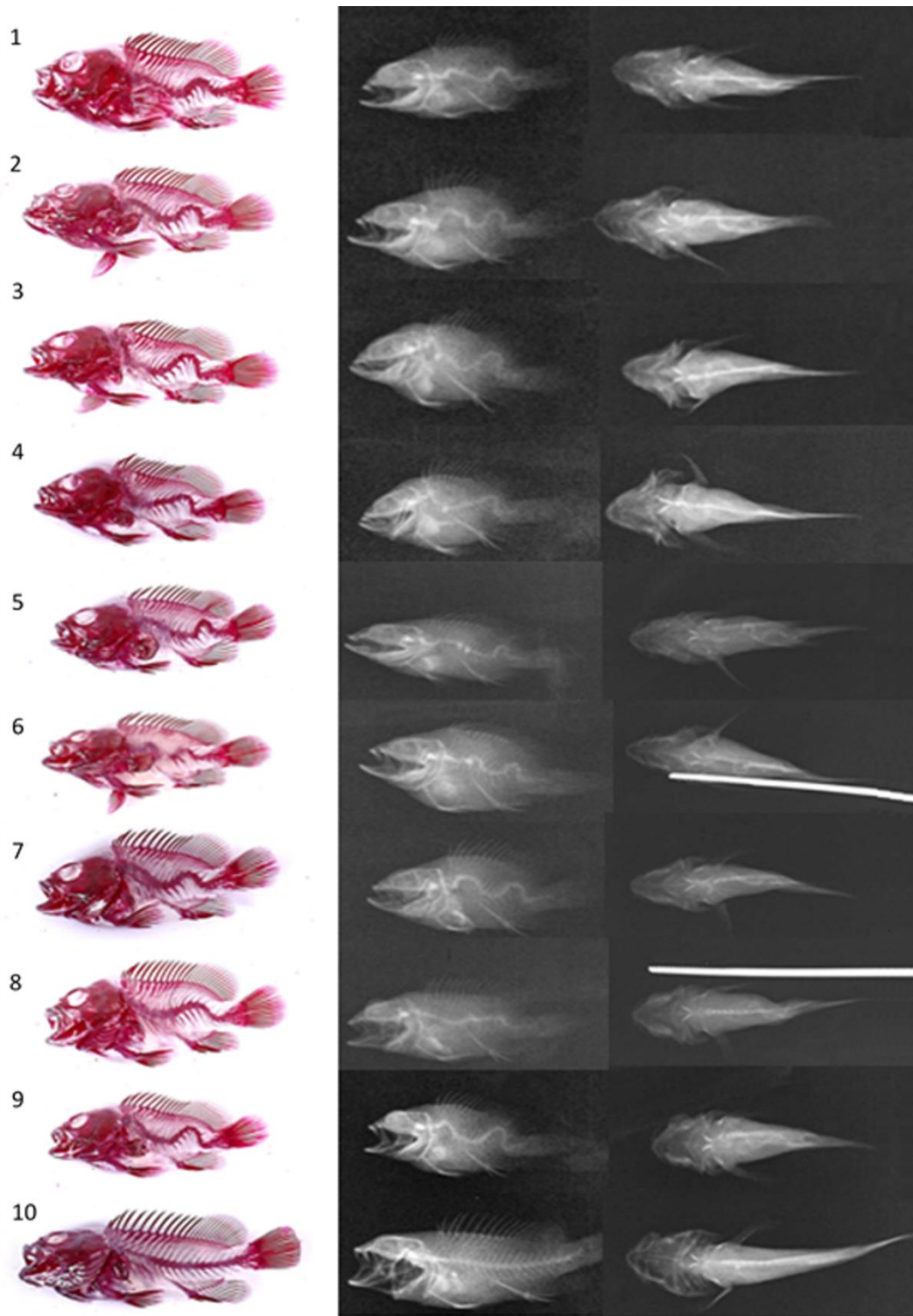


Figure 1. Diaphanization (left) and radiography (right) of dusky grouper *Epinephelus marginatus* juveniles with anomalies in the vertebral column (1-9) and with normal morphology (10). Scoliosis (1-9), kyphosis (1, 3, and 6), and lordosis (8-9).

groupers were fed with rotifers *Brachionus plicatilis* and *Artemia* sp., enriched with fatty acids and a commercial diet. The enrichment of food organisms

cannot rule out that nutritional deficiencies could cause skeletal anomalies in this study. More conclusive studies should be conducted to assess whether the

enrichment protocol used in this study is the most suitable for this species. The average temperature was kept at 25°C and the average salinity was kept at 35 during egg incubation, which are the averages of the species' natural environment and would not implicate stressful conditions (Kerber et al. 2012). Additionally, larviculture density can be considered low (4 larvae L⁻¹) and should not have any effects on the occurrence of anomalies (Boglione et al. 2009).

Skeletal anomalies such as kyphosis, lordosis, scoliosis, and spine fusion are pathologies related to bone reabsorption and remodeling that can also occur in response to a mechanical force (Boglione et al. 2013, Berillis 2015). Lordosis was attributed to the swimming effort of sea bass *Dicentrarchus labrax* juveniles (Divanach et al. 1997) and red sea bream *Pagrus major* with inflated swimbladder (Kihara et al. 2002). This relationship is due to the swimming effort larvae need to exert to maintain their position in the tank once the swim bladder is inflated.

The rationale for the high water speed in the larviculture tanks is to facilitate their cleaning. A more intense water movement drags the waste towards the water exit, reducing the need for siphoning the tanks in order to remove them. In this study, aeration (> 3 L min⁻¹) generated excessive air bubbles, resulting in intense water movement. Nevertheless, if such an intense water movement interferes with larval development, on the other hand, its lowering will implicate a significant reduction in the dissolved oxygen levels.

Establishing specific larviculture protocols to meet the needs of each species poses a great challenge. However, it is crucial to seek such improvements to reduce the development of skeletal anomalies. Significant research efforts have reduced them, but many recommended alternatives are not economically viable (Russo et al. 2011). Skeletal anomalies originated in the embryonic and larval stages (Koumoundouros et al. 1997). Therefore special care must be dedicated to these stages. The present study has demonstrated that the dusky grouper's larviculture protocol, as Kerber et al. (2012) proposed, must be reassessed to reduce anomalies. We recommend future studies that assess the impact of tank size in larviculture (depth, width, and length) associated with larval development.

Deformities are a complex mixture of different bone disorders that are poorly understood. This study did not identify the causes of skeletal anomalies in dusky grouper, but the high incidence of malformations may reflect culture problems due to rearing conditions that affect skeletal development. The register of the incidence of skeletal anomalies serves as a base for future studies on the occurrence of skeletal anomalies

and their possible causes in this species. Therefore, adjustments in the larviculture protocols will be necessary in order to minimize the occurrence of anomalies and the economic impacts on the production of dusky grouper juveniles.

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Data availability statement: The data supporting this study's findings are available from the corresponding author upon reasonable request.

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