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Deformities in reared cobia, *Rachycentron canadum* L. and grouper, *Epinephelus marginatus*, in São Paulo state coast, Brazil: case report

[Deformidades em cobias, *Rachycentron canadum* L, e garoupas, *Epinephelus marginatus*, cultivadas na costa do estado de São Paulo, Brasil: relato de caso]

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ABSTRACT

Skeletal deformities are frequent problems in cultured fish populations due mainly to unfavorable abiotic conditions, inappropriate nutrition, and genetic factors. These may cause erratic swimming, decrease of conversion rate, growth, and market value. The aim of this case report was to present the occurrence of deformities in juvenile cobia and groupers from an offshore marine farm in Ilhabela, São Paulo, Brazil. Nine cobias and seven juvenile groupers, that presented deformities, were euthanized and fixed in 10% formaldehyde. They were tagged, processed, and stained with "Alcian Blue" (AB) and "Alizarin Red-S" (ARS) for visualization of cartilage and bone tissue, respectively. After evisceration, radiographic examination was performed. The deformities observed in cobia were atrophy of the operculum, disorganization of the gill lamellae, deformity of the lower jaw, kyphosis, and scoliosis. Groupers presented lower jaw deformity and saddleback syndrome. The farm studied in this case report had poor management standards, fed commercial feed for freshwater herbivorous fish and cooked sardines, high inbreeding and absence of adequately trained professionals to manage these species. The sum of these factors must have directly influenced the appearance of these deformities. It is necessary to control this situation to minimize the incidence of malformations that have economic impacts to farmers.

Keywords: operculum atrophy, kyphosis, scoliosis, saddleback syndrome

RESUMO

As deformidades esqueléticas são problemas frequentes em populações de peixes cultivados devido, principalmente, a condições abióticas desfavoráveis, nutrição inapropriada e fatores genéticos. Estas podem causar natação errática, diminuição do índice de conversão, do crescimento e do valor no mercado. O objetivo deste relato de caso foi apresentar a ocorrência de deformidades em cobias e garoupas juvenis provenientes de uma fazenda marinha em sistema offshore, *em Ilhabela, São Paulo, Brasil. Nove cobias e sete garoupas juvenis, que apresentavam deformidades, foram sujeitos à eutanásia e fixados em formol a 10%. Os espécimes foram registrados, processados e corados com "Alcian Blue" (AB) e "Alizarin Red-S" (ARS) para visualização de tecido cartilaginoso e ósseo, respectivamente. Os peixes foram eviscerados para o exame radiográfico. As deformidades observadas em cobia foram: atrofia do opérculo, desorganização das lamelas branquiais, deformidade do maxilar inferior, cifose e escoliose. As garoupas apresentaram deformidade do maxilar inferior e síndrome de* Saddleback. *Os peixes deste relato de caso estiveram sob manejo deficiente, alimentados com ração comercial para peixes herbívoros de água doce e sardinhas cozinhadas, alta consanguinidade e ausência de profissionais adequadamente treinados para o manejo dessas espécies. A soma desses fatores influenciou diretamente na aparição dessas deformidades, precisando ser controlados para minimizar a incidência de malformações que tenham impacto econômico para os produtores.*

Palavras-chave: atrofia do opérculo, cifose, escoliose, síndrome de saddleback

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INTRODUCTION

Morphological deformities of skeleton, fins and scales occur in both wild and farmed fish during larval and juvenile development stages, and they are related to several physiological, environmental, genetic, xenobiotic, and nutritional factors (Lall & Lewis-McCrea, 2007). However, the prevalence of deformities in wild fish is usually very low (Diggles, 2013). Most of the skeletal deformities in fish include jaw, operculum, spinal (lordosis, kyphosis, scoliosis, and vertebrae fusion), and fin malformation such as saddleback syndrome, characterized by loss of one or more hard spines of the dorsal fin (Diggles, 2013). Vertebral deformities may occur when fish is exposed to unsuitable conditions during embryonic development such as low dissolved oxygen, water temperature changes, transportation, or incubation (Hattori *et al*., 2004). Deformities are also associated to physiological, xenobiotic, nutritional, and genetic factors during larval and juvenile development stages of cultured freshwater and marine fish (Cahu *et al*., 2003; Blogione *et al*., 2001; Ma *et al*., 2016).

These deformities have an impact on aquaculture reducing the market value of fish products (Nagano *et al*., 2007). In addition, skeletal deformities also decrease swimming ability, conversion index, growth, survival (Gavaia *et al*., 2009), and can lead to functional impairment (Noble *et al*., 2012). Thus, it is difficult for farmers to elucidate the causes of the deformities during the production of larval and juvenile fish (Haga *et al*., 2011). The aim of this report was to show the occurrence of deformities in juvenile cobia and grouper from an offshore fish farm in Brazil.

CASUISTRY

A high occurrence of deformities in cobia and grouper reared in offshore floating cages was observed in a hatchery in Ilhabela, São Paulo, Brazil in 2012. The specimens were fed during the larval stage with *Artemia* sp. enriched with vitamin C supplementation. Fingerlings aged 11dph (days post-hatch) were kept in 1m³ cages containing 100 animals each. Fishes were fed three times a day at 8% of biomass per day with commercial feed for freshwater fish, cooked sardines and vitamin supplement. Water temperature was 22°C.

A sample of nine juvenile cobia (length ranging from 14.6 to 17.8cm) and seven juvenile grouper (length ranging from 10.8 to 12.9cm) that showed functional and morphological changes were collected from the hatchery. These were anesthetized with benzocaine (100mg/L) and euthanatized by spinal cord section and fixed in 10% formalin. In addition, the specimens were examined, photographed and the morphological changes were recorded and classified according to the type and severity of the deformity. Two specimens of each species were processed according to the technique described by Taylor and Van Dyke (1985) and stained with "Alcian Blue" (AB) and “Alizarin Red-S" (ARS) for visualization of cartilaginous and bone tissues respectively. Fish were eviscerated for radiographic examination (X-Ray Siemens Tridoros Model 812E equipment), using high resolution Min-R 2000 (Kodak) Film, applying 36 kV to 200mA and 20-25mAs.

RESULTS AND DISCUSSION

The deformities observed in cobia were operculum atrophy, disorder of gill lamellae, lower jaw deformity, kyphosis, and scoliosis. Cobia with operculum atrophy also showed disorder of gill lamellae (Figure 1A and 1B). According to Boglino *et al*. (2012), live feed used for marine finfish larvae needs to be enriched with essential fatty acids to provide a balanced diet. However, larval stages in the hatchery were feed with *Artemia* sp. and supplemented only with vitamins. This protocol contains a reduced range of food for the larvae and could lead to nutritional imbalances or nutritional deficiencies (Watanabe, 1993), which suggests that some of the deformities such as kyphosis and scoliosis could be originated by nutritional requirements during the first stages.

Lower jaw deformities in cobia were also observed. Nagano *et al*. (2007) reported that the first anomalies observed during seven-band grouper developmental stages were jaw deformities, in particular the lack of supramaxilla or twisted upper and lower jaws. Some species such as striped trumpeter (*Latris lineata*) have requirements of highly unsaturated fatty acids (HUFA) and low concentrations in diets could be related to jaw malformation (Cobcroft *et al.*, 2001).

Nagano *et al*. (2007) showed there are several possible explanations for skeletal deformities in intensive aquaculture such as nutrition, streamflow, bacterial infection, light intensity, and water temperature. Furthermore, the farm studied in this case report had poor management standards, mainly due to fish were feed with commercial feed for herbivorous freshwater fish and cooked sardines, what means that fish were not received species-specific nutritional requirements. Cobia is a carnivorous pelagic fish and presented a relatively high protein requirement (Fraser & Davies, 2009). In addition, pathogens are possibly present in the fish used as feed. Hine & MacDiarmid (1997) mention that cooking food is likely to destroy fish pathogens in contaminated fish, although lightly cooked or raw fish may still contain viable organisms.

Some specimens showed operculum atrophy and disorder of gill lamellae, which can be related to nutritional requirements. Koumoundouros *et al*. (1997) mentions that vitamin C deficiency has frequently been related to the genesis of gill-cover shortening. It was observed that the vitamin C supplement used in the farm was expired. This may have resulted in loss of ascorbic acid by oxidation.

The specimens of grouper showed lower jaw deformity and saddleback syndrome. In addition, deformities in neural processes and proximal pterygiophore of saddleback grouper (Figure 1C, 1D e 1E) were observed in radiography and using AB and ARS staining. A high incidence of skeletal deformities has been described in the jaws and vertebral column during the larval and juvenile stages in seven-band grouper (*E. septemfasciatus*) and most of them presented abnormal maxilla curvature and lordosis (Nagano *et al*., 2007). Some studies developed by Cahu *et al*., (2003) reported that phospholipids, particularly phosphatidylinositol, reduced spinal malformations in larval *D. labrax*. In the present report, the examination of the skeletal deformities using radiography (Figure 1F) confirmed the deformities according to previous descriptions of saddleback syndrome in other fish species.

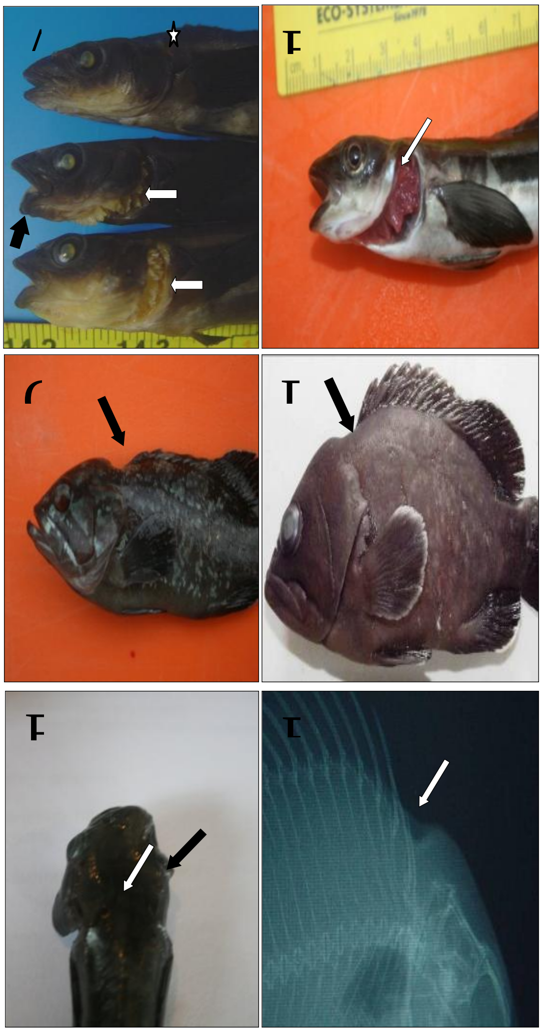

Figure 1. (A) Juveniles of *R. canadum* with operculum atrophy (white arrows), lower jaw deformity (black arrow) and kyphosis (white star). (B) Juvenile *R. canadum* with operculum atrophy and disorder of gill lamellae (white arrow). (C-D) Juveniles of *E. marginatus* with saddleback syndrome (black arrows). (E) Juvenile *E. marginatus* with saddleback syndrome (white arrow) and right operculum atrophy (black arrow) (dorsal view). (F) Radiography detail of saddleback syndrome with deformity of proximal pterygiophore (white arrow) in juvenile *E. marginatus.*

It is difficult to define the etiology of high rates of deformities in farmed fish due to the wide range of possible factors. However, nutritional and environmental factors must be controlled to minimize the occurrence of malformations to produce the minimum impact for farmers. Recent data provide more evidence of the important role of several dietary components for larvae in early stages (Cahu *et al*., 2003).

Some nutritional factors such as an expired supplement of vitamin C in the diet could have been responsible for the skeletal deformities, because of the instability and the loss of activity in the vitamin. Cahu *et al*. (2003) mentions that ascorbic acid supplied in the diet is crucial for fish larvae development, since most of the species are unable to synthesize vitamin C. In this report, causative factors of skeletal deformities could not be totally identified due to various external parameters in the hatcheries. However, the most likely is that nutritional and genetic factors were affecting skeletal development in these fish.

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REFERENCES

BLOGIONE, S.; GAGLIARDI, F.; SCARDI, M.; CATAUDELLA, S. Skeletal descriptors and quality assessment in larvae and post-larvae of wild-caught and hatchery-reared gilthead sea bream (*Sparus aurata* L. 1758). *Aquaculture*, v.192, p.1-22, 2001.

BOGLINO, A.; DARIAS, M.J.; ORTIZ-DELGADO, J.B. *et al*. Commercial products for *Artemia* enrichment affect growth performance, digestive system maturation, ossification and incidence of skeletal deformities in Senegalese sole (*Solea senegalensis*) larvae. *Aquaculture*, v.324-325, p.290-302, 2012.

CAHU C.; ZAMBONINO, J.; TAKEUCHI, T. Nutritional components affecting skeletal development in fish larvae. *Aquaculture*, v.227, p.245-258, 2003.

COBCROFT, J.M.; PANKHURST, P.M.; SADLER, J.; HART, P.R. Jaw development and malformation in cultured striped trumpeter *Latris lineata*. *Aquaculture*, v.199, p.267-282, 2001.

DIGGLES, B.K. Saddleback deformities in yellowfin bream, *Acanthopagrus australis* (Günther), from South East Queensland. *J. Fish. Dis*., v.36, p.521-527, 2013.

FRASER, T.W.; DAVIES, S.J. Review article nutritional requirements of cobia, *Rachycentron canadum* (Linnaeus): a review. *Aquacult. Res*., v.40, p.1219-1234, 2009.

GAVAIA, P.J.; DOMINGUES, S.; DINIS, M.T. *et al*. Comparing skeletal development of wild and hatchery-reared Senegalese sole (Solea senegalensis, Kaup 1858): evaluation in larval and postlarval stages. *Aquacult. Res*., v.40, p.1585-1593, 2009.

HAGA, Y.; DU, S.J.; SATOH, S. *et al*. Analysis of the mechanism of skeletal deformity in fish larvae using a vitamin A-induced bone deformity model. *Aquaculture*, v.315, p.26-33, 2011.

HATTORI, M.; SAWADA, Y.; KURATA, M. *et al*. Oxygen deficiency during somitogenesis causes centrum defects in red sea bream, Pagrus major (Temminck et Schlegel). *Aquacult. Res*., v.35, p.850-858, 2004.

HINE P.M.; MACDIARMID, S.C. Contamination of fish products: risks and prevention. *Rev. Sci. Tech*., v.16, p.135-145, 1997.

KOUMOUNDOUROS, G.; ORAN, G.; DIVANACH, P.; KENTOURI, L.M. The opercular complex deformity in intensive gilthead sea bream (*Sparus aurata* L.) larviculture. Moment of apparation and description. *Aquaculture*, v.156, p.165-177, 1997.

LALL, S.; LEWIS-MCCREA, L. Role of nutrients in skeletal metabolism and pathology in fish-An overview. *Aquaculture*, v.267, p.3-19, 2007.

MA, Z.; ZHENG, P.; GUO, H. *et al*. Jaw malformation of hatchery reared golden pompano Trachinotus ovatus (Linnaeus 1758) larvae. *Aquacult. Res*., v.47, p.1141-1149, 2016.

NAGANO, N.; HOZAWA, A.; FUJIKI, W. *et al*. Skeletal development and deformities in cultured larval and juvenile seven-band grouper, *Epinephelus septemfasciatus* (Thunberg). *Aquacult. Res*., v.38, p.121-130, 2007.

NOBLE, C.; JONES, H.A.; DAMSGÅRD, B. *et al*. Injuries and deformities in fish: their potential impacts upon aquacultural production and welfare. *Fish. Physiol. Biochem.*, v.38, p.61-83, 2012.

TAYLOR, W.R.; VAN DYKE, G.C. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium*, v.82, n.2, p.107-119, 1985.

WATANABE, T. Importance of docosahexaenoic acid in marine larval fish. *J. World. Aquacult. Soc*., v.24, p.152-161, 1993.