Thalassia Salentina
Thalassia Sal. 42 (2020), 59-74
ISSN 0563-3745, e-ISSN 1591-0725
DOI 10.1285/i15910725v42p59

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CASES OF ANOMALIES IN THE GOLDFISH *CARASSIUS AURATUS* COLLECTED FROM THE SOUTHERN MARSHES OF IRAQ

SUMMARY
Six morphological deformities were observed and examined in 6 specimens of the goldfish *Carassius auratus* collected from the southern marshes of Iraq, Basrah. These anomalies include: eye abnormalities (exophthalmos and enophthalmos), mouth deformity (dextral twisted mouth), ankylosis (fusion of vertebrae), lordosis (ventral curvature), pudgheadness (deformation of the front head bones) and scoliosis (lateral curvature). All cases were not fatal as they occurred in adult individuals. The paper discusses the possible causes for such deformities as well as the suitability of this kind of study for environmental monitoring.

INTRODUCTION

Fish might experience some factors that affect their development and such aspects were represented in the different types and levels of skeletal deformities that might lead to a decline in the growth and limit the survival of individuals. In the wild populations of fishes, naturally originated skeletal anomalies are present (Gavaia *et al*., 2009). The abnormalities in general can be either very severe in that they affect the fitness of the fishes, or slight and not impacting their survival. In the environment monitoring programs, knowing the frequency and occurrence of the skeletal anomalies considered the foundation of further investigations such as the valuation and checking of environmental pressures or rearing conditions for fishes (Yershov, 2008).

In general, skeletal development can be affected by external factors, which contain harmful environmental features at the larval and juvenile stages (Koumoundouros, 2010). These could be pollutants of different types or mutagens (Sfakianakis *et al*., 2006) or specific rearing or experimental conditions (Koumoundouros *et al*., 2001). On the other hand, many investigations have revealed that the reasons and mechanisms leading to individual imperfections are not always well known (Koumoundouros *et al*., 1997) as they may be a result of mutations disorderly the expression of various regulatory genes (Yamauchi *et al*., 2006).

The case of exophthalmos (also called exophthalmus, exophthalmia, proptosis, or exorbitismis), describes a protrusion of the eye out of the orbit. On the other hand, enophthalmos is backward displacement of the eyeball into the orbit or an abnormal sunken of the eyes into their sockets. In the enopthalmic eyes, a loss of normal ocular structure including retinal detachment and degeneration, the presence of eosinophilic within the vitreous area of the eye (Garcia-Abiado *et al*., 2006).

Spinal anomalies such as scoliosis (lateral curvature), lordosis (ventral curvature) and ankylosis (fusion of vertebrae) have been defined in many species both cultured and from the wild populations (Boglione *et al*., 1989). Lordosis is maybe the utmost well studied axis deformity in fishes. It can disturb every area of the vertebral axis. Scoliosis is most easily identifiable in live fish, with the best detection being from the dorsal or ventral side of the whole fish. Ankylosis, the changes resulting from deformation of the vertebral bodies, can be by compression, or a combination of compression and ankylosis (Boglione *et al*., 2013).

This study intends to describe the skeletal abnormalities in the goldfish *Carassius auratus* collected from the Al-Hammar Marsh, southern Iraq. The precise developmental stages at which the skeletal malformations initiate were not, however, known.

MATERIALS AND METHODS

Six specimens of teleosts fishes, *Carassius auratus* (family: Cyprinidae) ranging in total 120 – 330 mm showed spinal aberration and other structural deformities. Fishes were captured in the period from November 2013 to May 2014 in the waters of Al-Hammar Marsh, the largest southern marsh in southern Mesopotamia (Iraq) using different fishing gears such as gill nets and cast nets. Normal specimens were obtained from the same fishing lot at the same fishing locality to make a comparison. Body and fins were examined thoroughly for exterior parasites, malformations, amputations and any other morphological deformities. For the cases of lordosis, the length of the vertebral column from the anterior margin of the first vertebra to the posterior margin of the last vertebra was divided by fish total length to produce a ratio that is used to compare abnormal with normal fish. The angle of vertebral abnormality was measured from the centre of the anomaly, which in the present case was located in the caudal region by means of a digital protractor. Abdominal vertebrae were those situated immediately behind the skull and lacking haemal process. Caudal vertebrae are with haemal processes fused together forming haemal spine ventrally. Abdominal region is the region that includes abdominal vertebrae. Caudal region is the region that contains caudal vertebrae (Chapleau, 1988). The length of a vertebra is defined as parallel to the cranial-caudal axis, and the width is perpendicular. All measurements were made by the same person and instrument in order to increase the precision of measurements and reduce variability introduced by measurement error. The specimens were deposited in the fish collection of the College of Education, University of Basrah, Basrah, Iraq. In the laboratory, measurements were recorded to the nearest millimetre.

RESULTS

1. Exophthalmos and enophthalmos

One fish specimen (250 mm total length) showed eye deformities: exophthalmos (left eye) and enophthalmos (right eye) conditions (Fig. 1). Exophthalmic eye is large, normal and protruded globe bulging outside the orbit. The diameter of the exophthalmic eye is 30 mm. Enopthalmic eye exhibited loss of the globe and degeneration including the lens. The surface of this eye was concave, with skin cells and loose connective tissue fibres replacing the globe.

Figure 1. Cases of exophthalmos and enophthalmos in *Carassius auratus*, 250 mm TL.

2. Dextral twisted mouth

As the name proposes, the deformed specimen (230 mm total length) displays a twisting of both the upper and lower jaw (Fig. 2) evident when compared with a normally formed lower jaw. The whole anterior part of the head showed signed to have rotated 25° to the right. Due to the twisting of the upper and the lower jaws, the left side of the mouth appeared to be stretched, while the opening on the right side showed to be shorter and narrower than that of the left side. The mouth kept open due to this deformity.

Figure 2. Case of dextral twisted mouth in *Carassius auratus*, 230 mm TL.

3. Ankylosis

Externally, the body of the deformed fish (270 mm TL, Figs. 3a, b) looks short and stumpy, with normal head. Pectoral and caudal fins were normal. Lateral line slightly deformed posteriorly. Radiographs showed that vertebrae from both the abdominal and caudal regions of the vertebral column were involved in this deformity, with different levels of vertebral compression and ankylosis (Figs. 3b). The anomalies of the vertebrae from the anterior to posterior sides of the vertebral column are as follows: coalescence in abdominal vertebrae (V9-V10), deformed *centra* (V11-V13). In the caudal region, a severe deformed *centrum* was observed in V9, V10, while a mild deformed *centrum* was noticed in V11-V13. Minor abnormality such as wavy pleural ribs of the abdominal vertebrae 9-13 was observed. Because of the shortness of the base of the dorsal and anal fins, the interdigitation of the pterygiophores of both dorsal and anal fin appeared disturbed.

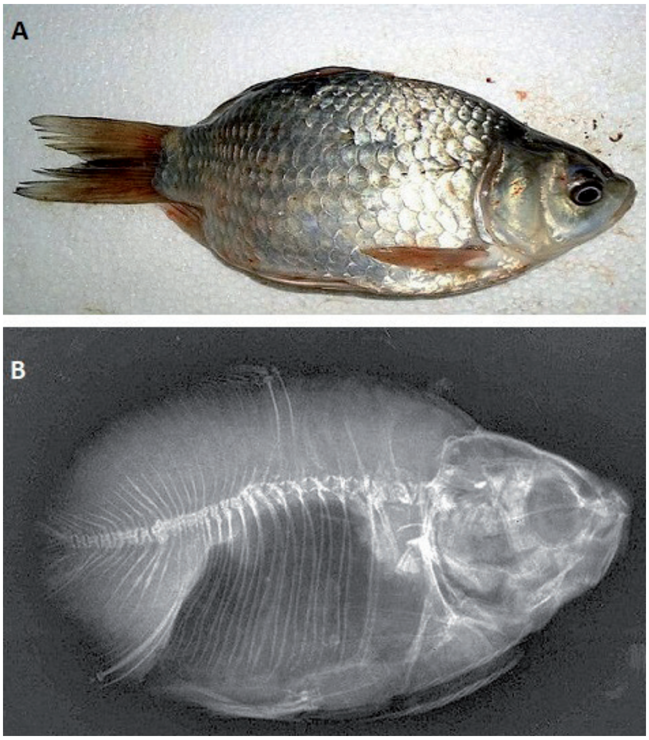

Figure 3. Case of ankylosis in *Carassius auratus*, 270 mm TL. A, external view; B, radiograph.

4. Lordosis

Morphologically, the body of the fish specimen with lordosis case (260 mm TL, Figs. 4a, b) does not show a clear deformity. The skeleton of the deformed specimen was compared with that of the normal. One flexion of the vertebral column at the caudal region was present. The caudal vertebrae 2-8 appeared to be involved in the lordosis incident. Elevation of the *centra* of the vertebrae started with the posterior part of V2 followed by complete rise of V3, reach curved vertebral column reached its highest point at V5 and V6 and then started to drop down at V7 and V8. The *centra* of V3-V8 were severely deformed. Vertebrae located toward the posterior end of the vertebral column showed a minor distortion.

The ratio of the vertebral column to the fish total length of the deformed specimen is 0.6, but it is 0.8 in the normal specimen. The haemal spines of the caudal vertebrae V4 – V8 showed slightly wavy and entangled. The value of the angle 'A' lying between the lines passing through the sides of the vertebral column and enclosing the curvature is 130°. The depth of the curvature of angle 'A' is 10.7 (Fig. 4b).

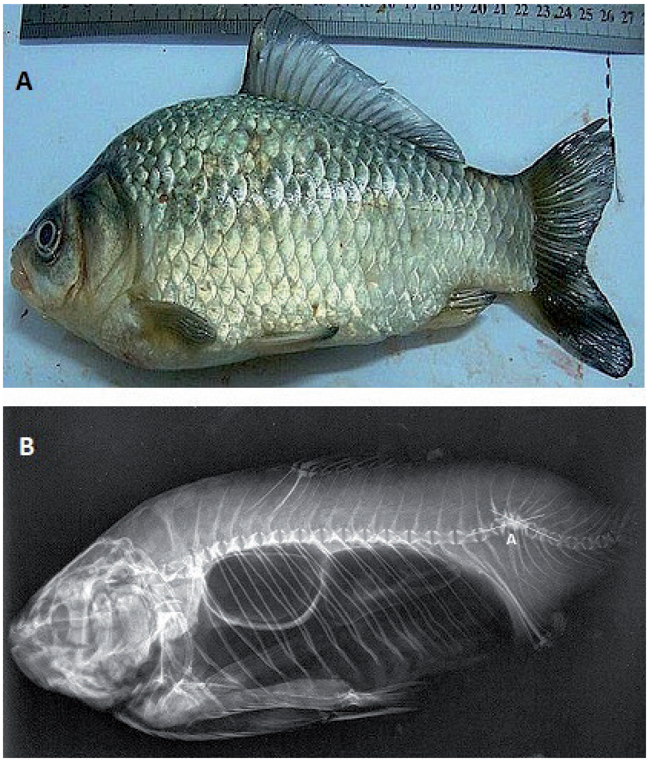

Figure 4. Case of lordosis in *Carassius auratus*, 260 mm TL. A, external view; B, radiograph.

5. Pugheadness

The pug-headed specimen had a 280 mm total length, 14.3 mm preorbital length and 24.2 mm postorbital length. This specimen is compared to normal fish having 285 mm total length, 18 mm preorbital length and 25 mm postorbital length.

The abnormal specimens were shown to have short neurocranium and a normal upper and lower jaws. The mouth was closed and the deformity has no significant effect on the mechanism of mouth operation. The shortening of the snout caused a steep forehead and brought nostrils close to the eye (Fig. 5). Internally, the vomer and parasphenoid were completely fused together, and displacement and/or curvature of the nasals, frontals, vomer, and palatines were observed. The premaxilla and maxilla appeared slightly deformed. It looks that all the bones anterior to the orbit were deformed and curved downward perpendicularly toward the mouth cavity. For these reasons, the forehead is upraised and steep. No other morphological deformities were observed.

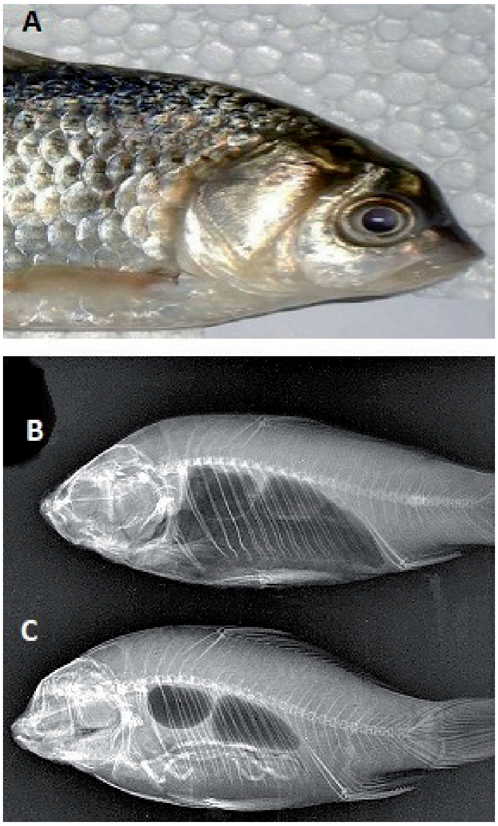

Figure 5. Case of pudgheadness in *Carassius auratus*, 280 mm TL. A, External view of the head; B, radiograph of normal specimen; C, radiograph of an abnormal specimen.

6. Scoliosis

The scoliosis was visible externally on the fish body (250 mm TL), with the spine curved sideway at two places, and compared with the normal specimen (Figs. 6a, b). The lateral line was disrupted posteriorly, no other external deformities were observed. The radiograph shows that both curves occur at the caudal vertebrae. The V8-V15 vertebrae were involved in the anterior curve and V16 V20 were intricate in the posterior curve. The angle between the two arms forming the angles A and B were 85° and 120° respectively. In the anterior curve, the *centra* of the V8 – V10 were severely deformed and joined together, while in the posterior curve, the *centra* of the vertebrae involved were slightly coalescent together.

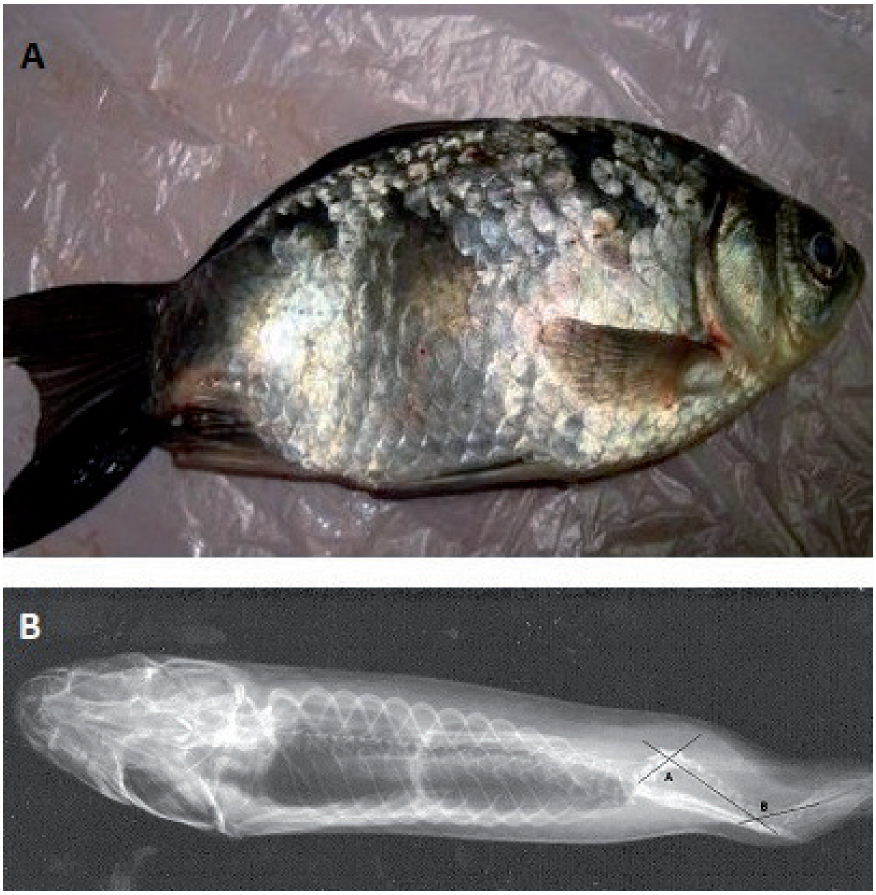

Figure 6. Case of scoliosis of *Carassius auratus*, 250 mm TL. A, external view; B, radiograph.

DISCUSSION

There is a considerable sum of information on wild fish deformities (Jawad and Liu, 2015). Investigators have examined both genetic (Ishikawa, 1990) and epigenetic causes as a possible cause of such anomalies (Boglione *et al.*, 1995), as well as environmental influences such as temperature, light, salinity, pH, low oxygen concentrations, inadequate hydrodynamic conditions and parasites (Gavaia *et al.*, 2009).

In the present deformed specimen, the anomalies represent cases of exophthalmos and enophthalmos, dextral twisted mouth, ankylosis, lordosis, scoliosis and pugheadness.

Among the causes that stand behind the cases of exophthalmos and enophthalmos is the gas bubble disease (for exophthalmos) (Noble *et al.*, 2012). Such disease can form when the sum of the dissolved gas pressures exceeds the sum of the hydrostatic pressure-simply put super-saturation of gas in the water (Bouck, 1980). The other possible cause could be the result of corneal trauma followed by secondary fungal and bacterial infection because of destruction of the eyes normal protective barriers (Eagle, 1999). High movement during warmer temperatures are possibly to increase interaction between individuals, which rises the probability of corneal strain. The formation of secondary fungal/bacterial infection would also be normal at higher temperatures (Garcia-Abiado *et al.*, 2006). Silverside (1976) performed experiments to show the effects of different levels of several insecticides on the development of the embryos of *Menidia menidia*.

At this stage, it is not possible to check whether the gas bubble disease is the causative agent in the case of the fish specimen with the exophthalmos condition because such confirmation needs several experiments to support this conclusion and a suggested future research needs to be done on this issue to reveal the contribution of the gas bubble disease to this anomaly.

The other two options of causative agents, the corneal trauma and the secondary fungal infection and the pollution by insecticides seems possible in the case of the fish in the present study. In the marsh areas, water temperature is high during most of the days of the year (Hamdan *et al.*, 2010). In addition, the marsh areas in general are shallow enough to have fish individuals of different species crowded in certain location (Al-Hilli *et al.*, 2009). Besides, the water of the marshes is well contained with different species fungus that could lead to a secondary infection of the eye of the fish (Al-Saadoon and Al-Dossary, 2014). It is not unusual to find location in the marsh areas free of insecticides pollutants or pollutants of any type (Salman, 2011). The insecticides originated from the agricultural lands that the Euphrates River pass through before entering the great marsh area (DouAbul *et al.*, 1988).

Damage of eyesight during larval development may be harmful because many species of fish at larval stage are visual predators (Porter and Theilacker, 1999). Garcia-Abiado *et al.* (2006) noted that the damage or loss of the left or right eye or both eyes may have different consequences to the survival and viability of the fish. The left eye inspects familiar part of the environment and the right eye to inspect for potentially dangerous stimuli such as the presence of a predator (Bisazza and De Santi, 2003; Sovrano, 2004). Any eye damage or loss will jeopardise the effective antipredator behaviour, where fish exhibit rapid burst of swimming as a panic response (Quist and Guy, 2004).

Fraser and De Nys (2005) have suggested that the twisting of both upper and lower jaws can be as an outcome of the shortened or twisted dentary and angular bone of one side of the bilateral jaws structure. The result is a migration of the symphysial joint in the direction of the deformed half. The other half of the jaw structure appears to be pulled to follow to the deformed side.

The twisting of the jaw can be in either a left or right direction. The present case of jaws twisting is similar to those reported for other fish species, *Lates calcarifer* by Fraser and De Nys (2005) and *Hippoglossus hippoglossus* by Morrison and Macdonald (1995).

Dietary ascorbic acid (Vitamin C) is widely recognised as a controlling factor in the development of skeletal deformities in adult and juvenile teleosts (Cahu *et al*., 2003). Vitamin C is vital for the production of collagen and hence correct bone formation. Chavez De Martinez (1990) observed jaws twisting in *Cichlasoma urophthalmus* fry fed a diet deficient in ascorbic acid for 49 days, while the torsion created by the jaw musculature on poorly formed bones is the mechanism responsible for the development of abnormal jaw structures (Swan, 1968). This offerings a possibility for further work exploring how dietary ascorbic acid affecting the development of jaw in the species in question and other freshwater fish species of Iraq as there is a significant lack of investigations in this field.

Ytteborg *et al*. (2012) suggested that there are 4 stages that characterized vertebral fusion (ankylosis). Such stages could result in the spinal fusion case in the specimen of *C. auratus*. Among these stages is the increase of disorganized and proliferating cells at the growth zones extended along the rims of fusing vertebral bodies. It is possible that the deformed specimen of *C. auratus* has confronted unfavourable environmental influences that might cause this type of vertebral abnormality. Since the specimen of *C. auratus* was an adult, the deformation was not fatal, but it definitely influenced the mobility in some way.

Lack of certain nutritional components such as phospholipids might be considered a possible cause for the skeletal deformities in *C. auratus*. Kanazawa *et al*. (1981) showed that phospholipids reduced vertebral deformities in larvae of *Plecoglossus altivelis* and phosphatidylinositol reduced spinal malformations in larval *D. labrax* (Cahu *et al*., 2003). On the other hand, excess of phospholipids induced severe skeletal malformations in larval *D. labrax* (Villeneuve *et al*., 2005). Mosa (2012) and Khidhir *et al*. (2013) have shown that the level of lipids the freshwater fish species of Iraq is low in comparison with the international standard for healthy fish species.

The morphological changes in the case of lordosis shown in the specimen of *C. auratus* are related anterior-posterior (i.e. cranial-caudal) compression along the spine. Structural indication is present in the X-ray show that the normal amphicoelous (hour-glass) shape of vertebrae is distorted so that vertebral height is reduced on the convex and is greater on the concave side of curvature. Similar changes were observed in *Poecilia reticulata* by Gorman *et al*. (2010). They suggested that the observed changes in vertebral bone structure may be caused by either (1) distortion of normal vertebral shape or (2) active remodelling of vertebral osteoid bone as a consequence of extrinsic forces. Several studies have demonstrated that bone modelling may be affected by water elevated Oxygen levels through the impact on bone mineral composition (Martens *et al*. 2006). The southern marshes of Iraq showed variation in water temperature during the years will induce similar variation in oxygen level in water, with extreme low level in summer time when temperature (Al-Saad *et al*., 2010). Any anomaly in the shape of the vertebrae which contains remodelling will have an immediate influence on the swimming capability of the fish and its survival (Koumoundouros *et al*., 1997), and there was a noteworthy relationship between the lordosis severity and swimming performance in sea bass (*Dicentrarchus labrax*) at least in juveniles (Peruzzi *et al*., 2007).

In teleosts, the pughead deformity case can be envisaged in four stages: normal, primary, secondary and tertiary stage (*sensu* Hickey *et al*., 1977). The present case of mild pughead anomalies in *C. auratus* represent the secondary stage in the system proposed by Hickey *et al*. (1977). This case is similar to the cases reported for *Bodianus rufus* from the Brazilian rocky reef by Macieira and Joyeux (2007) and *Salmo salar* from a hatchery in Norway by Jawad *et al*. (2014). In the forehead area of *C. auratus* examined, the ethmoid seem bent downward and backward causing mild blockage to the mouth. On other hand, the deformity left the mouth open a case that might seems less severe as the mouth at least open allowing feeding activity. The displacement of the posterior part of the skull shown in the x-ray of *C. auratus* might have a direct effect on the brain. Since the preorbital area was reduced very much and the nasal openings were lost in this specimen, the nasal organs and probably the olfactory nerve was lost too. Fishes with head deformity may fail the ability to breathe and feed, which in turn became unable to compete for obtaining food (Bortone, 1972; Hickey, 1973).

The reasons of the observed pughead anomaly in the specimen examined are unknown, but they probably arise during early development (Cobcroft *et al*. 2001). The survival rate of abnormal fish, especially during the early ontological stages in the wild, is unknown (Bueno *et al*., 2015). Genetic and epigenetic factors such as mutations or recombination of genes and exposure to contaminants such as cadmium, zinc, lead, mercury could cause pughead deformity (Dahlberg, 1970; Slooff, 1982).

The economic outcomes of the deformities explained in the present study are important in terms of reduced growth and ultimately weight and a much reduced value per kg fish captured. Therefore, further efforts such as improvements in management of the fisheries industries should be made to explore the various aetiological causes of the deformities.

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