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Skeletal deformities in *Barbus xanthos* (Teleostei: Cyprinidae) collected from the Dalaman River in southwestern Turkey

by

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Abstract

In this study, skeletal anomalies such as vertebral centra deformation, lordosis (ventral curvature), and consecutive repetition of lordosis and kyphosis were examined in specimens of *Barbus xanthos* (Güçlü, Kalaycı, Küçük & Turan, 2020) collected from the Dalaman River, southwestern Turkey. Abnormalities of the vertebral column were observed in both thoracic and caudal vertebrae. Cases of lordosis and consecutive repetition of lordosis and kyphosis showed varying degrees of severity. Specimens with consecutive repetition of lordosis showed the most acute deformities among the cases studied, as they revealed complicated incidences of skeletal anomalies. None of the cases were fatal as they occurred in adult individuals. This study discusses the possible causes of such deformities and the usefulness of this type of study in environmental monitoring.

Key words: vertebral deformity, lordosis, kyphosis, pollution, environment

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1. Introduction

Fisheries biologists, ichthyologists and aquaculturists have recently become more concerned about skeletal anomalies of wild and reared fish (Kužir et al. 2015). Many incidences of different types of deformities (Afonso et al. 2000; Sato 2006; Jawad et al. 2017a; Jawad & Ibrahim 2018) have been described for different fish species inhabiting different environments (Iwasaki et al. 2018). It has been found that these abnormalities can affect various parts of the fish body (De La Cruz-Aguero & Perezgomez-Alvarez 2001). Such anomalies have shown to have adverse effects on the life of fish as well as minimize the commercial value of fisheries (Raja et al. 2016; Majeed et al. 2018). For fish in the wild, skeletal anomalies, which can occur at any stage of the fish life cycle, can cause complications, for example in protecting their territories (Sato 2006; Majeed et al. 2018), competing for a mate (Sato 2006), and reduction in fisheries production (Noble et al. 2012). In aquaculture facilities, such deformities may affect animals by reducing their growth rates (Hansen et al. 2010), impairing their feeding ability (López-Olmeda et al. 2012; Okamura et al. 2018), increasing the risk of infection (Janakiram et al. 2018) and increasing mortality rates (Jara et al. 2017). Furthermore, these undesirable effects of skeletal aberrations will result in economic decline of fish farms (Boglione 2013; Yıldırım et al. 2014).

Skeletal aberrations frequently observed and described in several fish groups are centra deformation, kyphosis, and lordosis. Such anomalies can occur at either mild or severe levels in both aquaculture and wild individuals (Jawad & Ibrahim 2018; Näslund & Jawad 2021). Vertebral centra deformation can be mild or severe, and in the latter case the fish will have difficulty swimming and can lead to compression or a combination of compression and fusion of vertebrae (Witten et al. 2006). Lordosis is the most frequently described axis deformity in fish. It can be found in any region of the vertebral column. In the pre-hemal region of the column, it is called pre-hemal lordosis and is associated with failure to inflate the swim bladder (Chatain 1994). Other types include hemal lordosis, which is a common anomaly found in fish (Jawad et al. 2014; Fjelldal et al. 2009), cranial lordosis (i.e. affecting the most anterior vertebrae) and caudal lordosis (affecting the centra of the caudal peduncle). Kyphosis is considered less common than lordosis (Boglione et al. 2013). As in the case of lordosis, it can occur in pre-hemal and hemal locations (Boglione et al. 1995).

Studies on anomalies in freshwater fish species in Turkey are very limited. To our knowledge, the work by Jawad and Oktener (2007) on *Planiliza abu* collected from the Atatürk Dam Lake, the experimental work by Çelik et al. (2012) on the common carp, *Cyprinus carpio*, and the work by Innal et al. (2019) on *Barbus pergamonensis* from the Dalaman Stream are the only available studies on fish skeletal abnormalities in Turkey. Therefore, the present study adds to what is known regarding the prevalence of abnormalities observed so far in freshwaters of Turkey and describes cases of vertebral centra deformities, lordosis and consecutive repetition of lordosis and kyphosis anomalies observed in 9 specimens of *Barbus xanthos* (Güçlü, Kalaycı, Küçük & Turan, 2020) collected from the Dalaman River–Yusufça irrigation regulator (Çavdır-Burdur), Turkey. The availability of such information is very important for aquaculturists, fisheries biologists and environmentalists.

2. Materials and methods

2.1. Sampling locality

The study was carried out in the Dalaman River–Yusufça irrigation regulator (Çavdır-Burdur; Turkey; 37°13′33.87′′N; 29°32′55.79′′E). The Dalaman River originates in the Yeşilgöl Mountains in the south of Gölhisar (Burdur) and flows into the Mediterranean Sea at the borders of the Dalaman district (Fethiye; Fig. 1). The Dalaman River forms the border between the Mediterranean and Aegean regions (Turkey). The total length of the river is 229 km. The depth of the sampling location is approximately 1.5 m.

2.2. Fish samples

Sampling was carried out along a 50 m transect in June 2010 using electrofishing equipment. A total of 28 *Barbus xanthos* individuals were caught from the Dalaman River–Yusufça irrigation regulator (Figs

Figure 1

Map showing the fish sampling location

2a, b); *B. xanthos* is the only fish species living in the habitat selected for fishing. Nine specimens measuring 70.96–141.26 mm SL showed different skeletal abnormalities revealed by X-ray examination. A healthy specimen (175.2 mm SL) was obtained for reference (Fig. 3). The body and fins of the specimens were carefully examined for malformations, amputations and any other morphological anomalies. The specimens were fixed in 4% formaldehyde solution for further examination and deposited in the Inland Fish Collection, Eğirdir Fisheries Faculty of Isparta University of Applied Sciences under catalogue number IFC-ESUF 03-0481. The skeletons of both normal and abnormal specimens were examined using a mammography unit (Siemens/Mammomat Inspiration 2013) at an exposure time of 2.1 seconds, available at Meddem Hospital, Department of Radiology, Mammography Section, Isparta, Turkey.

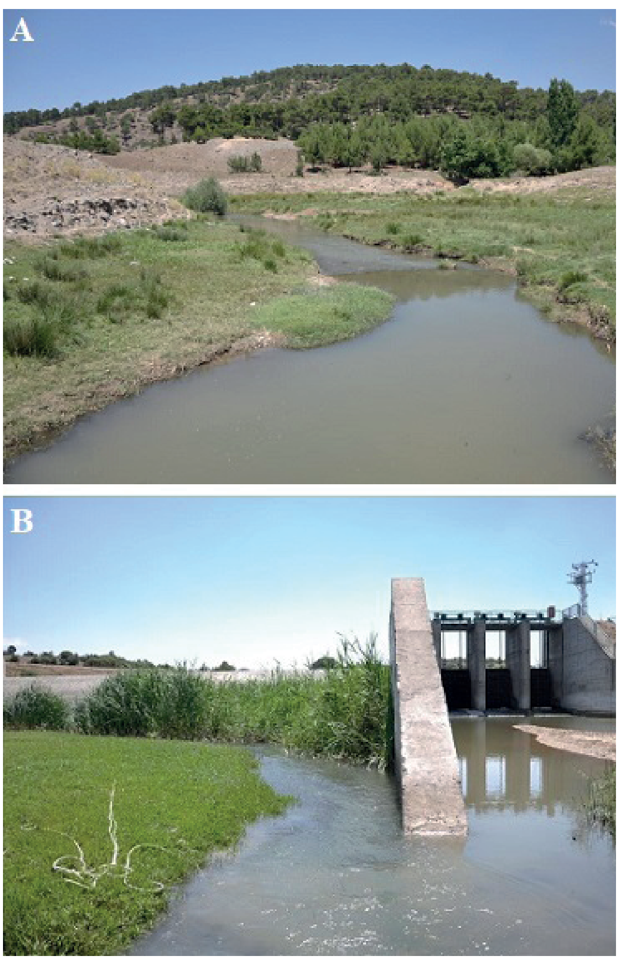

Figure 2

A: Yusufça irrigation regulator (Dalaman River, Çavdır-Burdur); B – Yusufça irrigation regulator (Dalaman River, Çavdır-Burdur)

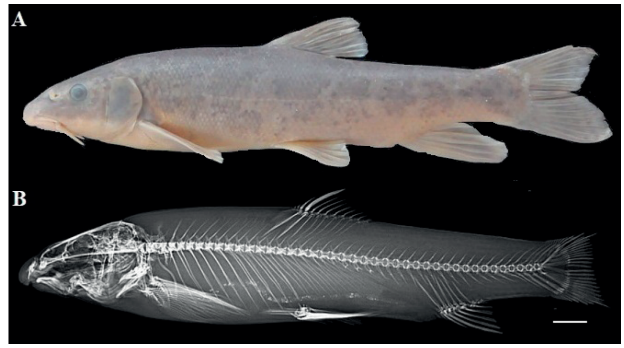

Figure 3

Normal specimen of *Barbus xanthos* 175.2 mm SL. A – whole fish; B – radiograph; scale bar = 1 cm

2.3. Morphological measurements and statistical analysis

To assess the severity of lordotic and kyphotic cases, the angle formed between the ascending and descending parts of the vertebral column was measured. The lower value of this angle indicates a severe case, while a high value indicates mild cases. The angle of vertebral deformation was measured from the center of the deformity by means of a digital protractor.

3. Results

The examination of *B. xanthos* specimens revealed that one specimen showed vertebral centra deformation (127.74 mm SL), four specimens showed lordosis (70.96, 80.56, 141.26, 81.05 mm SL) and four specimens (71.12, 81.53, 91.52, 98.9 mm SL) showed a consecutive repetition of lordosis and kyphosis.

3.1. Case of centra deformation

One specimen (127.74 mm SL) appeared externally normal compared to an abnormal specimen of nearly comparable size (Fig. 4a). This specimen had a single deformity, i.e. the centrum of the third caudal vertebra appeared deformed and displaced (Fig. 4b). In addition, the neural and hemal spines pointed more posteriorly and anteriorly, respectively. No other anomalies were observed in this specimen.

3.2. Cases of lordosis

The radiograph of the first abnormal lordotic specimen shows 19 vertebrae (10 thoracic and 9 caudal vertebrae) involved in the lordotic arch of the

Figure 4

Specimen of *Barbus xanthos* showing a vertebral centra deformity. A – whole fish 127.74 mm SL; B – radiograph of *Barbus xanthos* 127.74 mm SL showing a vertebral centra deformity; scale bar = 1 cm

deformed specimen (Figs 5 & 6a). The descending part of the lordotic arch contains five thoracic and five caudal vertebrae, while the ascending part of the arch contains nine caudal vertebrae. The value of the lordotic angle "A" was 152°.

In the second abnormal lordotic specimen (Figs 5 & 6b), 11 vertebrae were involved in the formation

Figure 5

Specimens of *Barbus xanthos* showing varying degrees of lordosis. A – 141.26 mm SL; B – 81.05 mm SL; C – 80.56 mm SL; D – 70.96 mm SL; scale bar = 1 cm

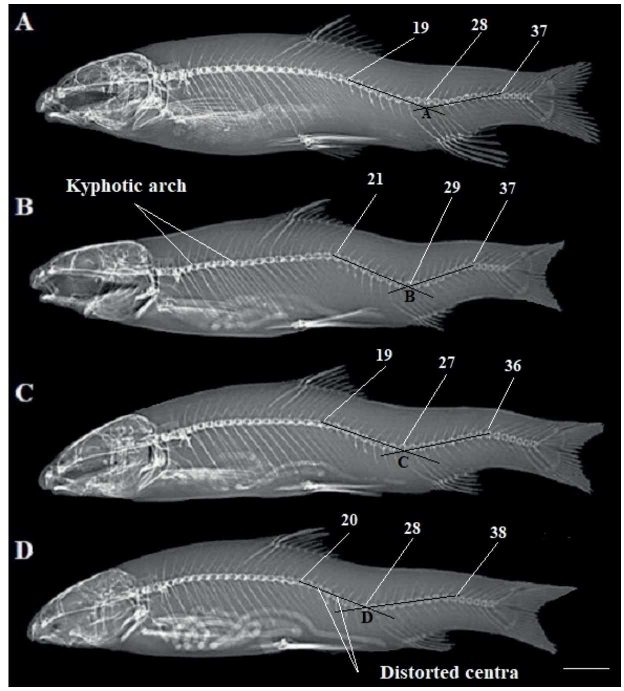

Figure 6

Radiograph of *Barbus xanthos* specimens showing varying degrees of lordosis. A – 141.26 mm SL; B – 81.05 mm SL; C – 80.56 mm SL; D – 70.96 mm SL; scale bar = 1 cm

of the lordotic arch. The descending part of the arch consisted of three and 14 thoracic and caudal vertebrae, respectively. The ascending part of the lordotic arch consisted of eight caudal vertebrae. The value of the lordotic angle "B" was 140°. Another slight anomaly observed in this specimen was the presence of a low kyphotic curve formed by thoracic vertebrae 5–10. No other skeletal anomalies were observed.

The lordotic arch of the third abnormal specimen, as revealed by the radiograph (Figs 5 & 6c), consisted of 18 vertebrae. The ascending part of the arch consisted of nine vertebrae, including six and three thoracic and caudal vertebrae, respectively. The value of the lordotic angle "C" was 148°. No other skeletal deformities were observed.

In the fourth lordotic specimen, the lordotic arch contained 19 vertebrae. The descending part of the arch consisted of nine vertebrae, including six and three thoracic and caudal vertebrae, respectively. The ascending part consisted of 10 caudal vertebrae. The lordotic angle "D" was 153°. Some of the centra of the vertebrae forming the descending part of the lordotic arch appeared distorted.

3.3. Case of consecutive repetition of lordosis and kyphosis

Four specimens with dimensions of 71.12, 81.53, 91.52, and 98.9 mm SL fell within this criterion of skeletal deformities (Figs 7 and 8). They showed different degrees of severity of both lordosis and kyphosis anomalies. Radiographs of these four abnormal specimens revealed that specimens "A" and "C" shown in Figs 8a and 8c had one lordotic and kyphotic arch, while specimens "D" (Fig. 8 d) had two kyphotic and one lordotic arches. Specimen "B" (Fig. 8b) appeared to be the most affected as it had four arches, two of each kyphotic and lordotic arch. One lordotic and one kyphotic arch were observed in the thoracic region of the vertebral column of specimens "B" and "D", respectively.

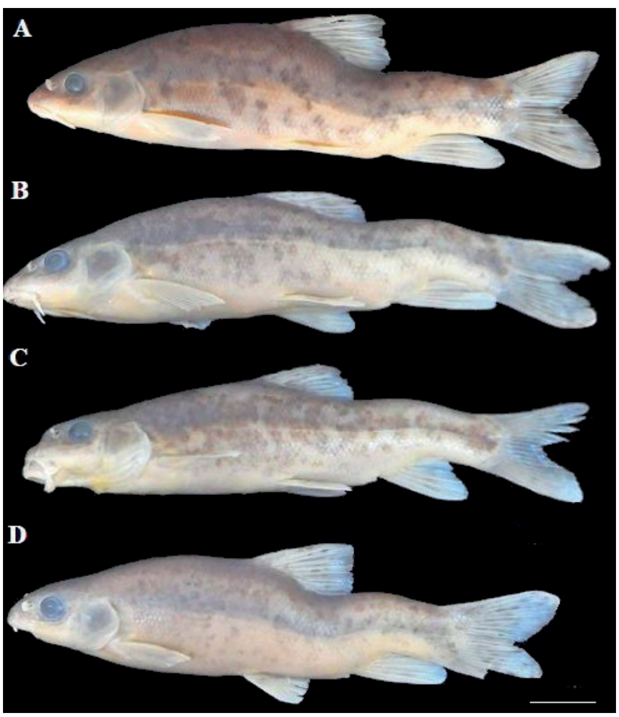

Figure 7

Specimens of *Barbus xanthos* showing varying degrees of consecutive repetition of lordosis and kyphosis. A – 98.9 mm SL; B – 91.52 mm SL; C – 81.53; D – 71.12 mm SL; scale bar = 1 cm

In the first lordotic arch observed in specimen "A" (Fig. 8a), nine and six vertebrae were involved in the formation of this arch (nine vertebrae involved in the descending part and six vertebrae in the ascending part). The descending part of this arch contained seven and two thoracic and caudal vertebrae, respectively,

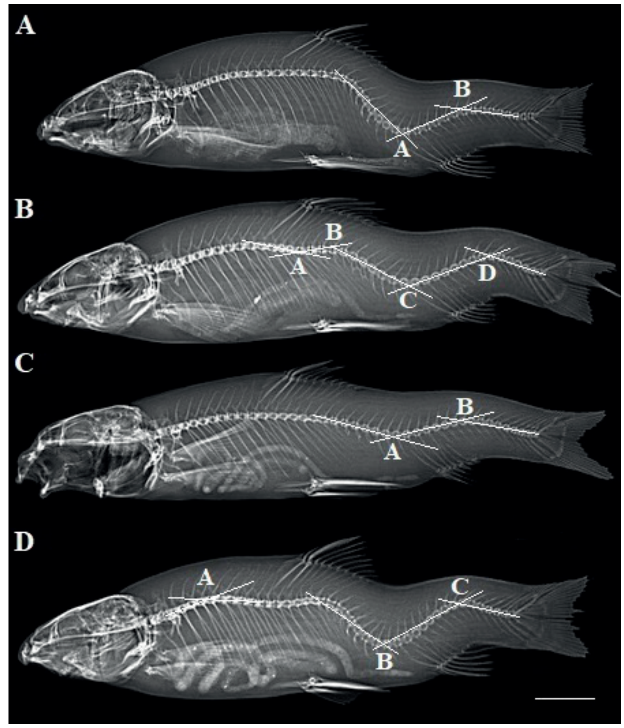

Figure 8

Radiograph of *Barbus xanthos* specimens showing varying degrees of consecutive repetition of lordosis and kyphosis. A – 98.9 mm SL; B – 91.52 mm SL; C – 81.53; D – 71.12 mm SL; scale bar = 1 cm

while all the vertebrae contained in the ascending part were caudal vertebrae. The kyphotic arch of specimen "A" consisted of 13 caudal vertebrae, including six and seven vertebrae forming the ascending and descending parts of the arch. The lordotic and kyphotic angles were 111° and 150° for the angles "A" and B" respectively.

The second lordotic-kyphotic deformed specimen (Fig. 8b) showed four arches, two lordotic and two kyphotic arches. The first lordotic arch consisted of 11 vertebrae. The descending and ascending parts of this arch contained seven and six thoracic vertebrae, respectively. The value of the lordotic angle "A" in this arch was 159°. The next arch in this species toward the tail is the kyphotic arch. This arch consisted of 11 vertebrae. The ascending and descending parts of this arch contain four and seven vertebrae, respectively. The seven vertebrae of the descending part contain five thoracic and two caudal vertebrae. The value of the kyphotic angle "B" in this arch was 139°. The third arch in this abnormal specimen is the lordotic arch, which is formed by 14 vertebrae, seven vertebrae in the ascending part, and another seven vertebrae in the descending part. The vertebrae of the descending part consisted of five thoracic and two caudal vertebrae. The value of the lordotic angle "C" was 132°. The fourth arch is kyphotic and consisted of 15 caudal vertebrae, with seven and eight vertebrae in the ascending and descending parts, respectively. The value of the kyphotic arch angle "D" was 141°.

There were two arches in the 3rd abnormal specimen (Fig. 8c). The first arch was lordotic and consisted of 13 vertebrae, with six and one thoracic and caudal vertebrae, respectively, in the descending part, and six caudal vertebrae in the ascending part. The second arch was kyphotic and consisted of 16 caudal vertebrae, with six and 10 vertebrae in the ascending and descending parts of this arch. The value of the lordotic angle "A" is 150° and the kyphotic angle "B" is 160°.

The fourth deformed specimen with this type of anomaly had three arches, one and two lordotic and kyphotic arches, respectively (Fig. 8d). The first kyphotic arch consisted of 11 thoracic vertebrae, with five and six vertebrae in the ascending and descending parts of this arch, respectively. The value of the kyphotic angle "A" of this arch was 160°. The second arch was lordotic and consisted of 18 vertebrae, with nine vertebrae in the ascending and descending parts, with an angle "B" of 150°. The descending part contained seven and two thoracic and caudal vertebrae, respectively. The third arch in this specimen was kyphotic and contained 18 caudal vertebrae, with the angle of the kyphotic arch being 140°.

4. Discussion

This is the first study presenting the occurrence and types of skeletal deformities in adult specimens of the teleost fish species *Barbus xanthos* collected from southwestern Anatolia, Turkey. The objective was to categorize skeletal deformities in the examined specimens and to determine a likely relationship between these abnormalities and environmental factors.

There are a number of studies on deformities in wild fish (Divanach et al. 1997; Jawad et al. 2013; Jawad & Liu 2015). Scientists have considered both genetic (Ishikawa 1990) and epigenetic aspects as a plausible source of such abnormalities (Boglione et al. 1995), in addition to the effects of environmental factors such as temperature, light, salinity, pH, low oxygen concentrations, inadequate hydrodynamic conditions, and parasites (Gavaia et al. 2009; Chatain 1994).

Ytteborg et al. (2012) described four stages of vertebral fusions. These stages can result in the vertebral central deformity observed in one specimen of *B. xanthos* examined in the present study. They are growth stages of disorganized and multiplying cells in the growth zones extending along the edges of adjoining vertebral bodies. Numerous mammalian studies suggest that an imbalance between cell death and cell proliferation may cause malformations.

Lack of specific nutrients, such as phospholipids, was considered a possible cause of the vertebral centra deformity observed in the present study. Kanazawa et al. (1981) showed that phospholipids reduce vertebral aberrations in larvae of *Plecoglossus altivelis* and phosphatidylinositol reduces spinal deformities in *D. labrax* larvae (Cahu et al. 2003). Excess phospholipids induce acute skeletal anomalies in *D. labrax* larvae (Villeneuve et al. 2005).

The divergence in the shape of the vertebral column in the form of lordosis and kyphosis demonstrated by several specimens of *B. xanthos* in the present study is associated with anteroposterior (i.e. cranial-caudal) compression along the spine. X-rays of the deformed specimens (Figs 6 and 8) show that the normal amphicoelous (hourglass) shape of vertebrae is distorted so that the vertebral height is shorter on the convex side and greater on the concave side of the curvature. In addition, vertebrae located approximately at the lower center of the curvature (in the case of the lordotic arch) are wedged, so that the length on the concave side of the curve is reduced relative to the convex length. Gorman et al. (2010) observed comparable disparities in *Poecilia reticulata*. They concluded that deviations in vertebral bone structure may be due to either (1) distortion of normal vertebral shape or (2) active remodeling of the vertebral osteoid bone under extrinsic forces. They added that vertebral development in fish is different from that in other animal models. *P. reticulata,* which they studied*,* has vertebrae composed of acellular bones (i.e. lacking entrenched osteocytes and formed by intramembranous ossification; reviewed in Witten & Huysseune 2009). Thus, future surveys of vertebral wedging in *B. xanthos* and other fish species exhibiting lordosis and kyphosis should test cellular activity in the intervertebral region (Inohaya et al. 2007).

The consecutive presence of lordosis and kyphosis (L–K) observed in the four specimens of *B. xanthos* examined in the present study may be genetically determined. Such a comment was made by Afonso et al. (2000) on a similar case they observed in *Sparus aurata*. The source of lordosis and kyphosis was not investigated in the present study and thus a genetic connection could not be hypothesized. Therefore, a vertebral anomaly was proposed, namely a consecutive repetition of lordosis and kyphosis (L–K syndrome).

The cases of lordosis, kyphosis, and the consecutive repetition of lordosis and kyphosis examined in this study matched similar incidences described in different fish species collected from the Turkish waters. Jawad & Öktener (2007) studied these abnormalities in *Liza* (= *Planiliza*) *abu* obtained from the Ataturk Dam Lake. Jawad et al. (2017b) determined cases of lordosis, kyphosis and the consecutive repetition of lordosis and kyphosis in *Atherina boyeri* collected from the Homa Lagoon, and Izmir, and in *Mullus barbatus* (Jawad & Akyol 2018) and *Mugil cephalus* (Jawad et al 2017a) collected from the northern Aegean Sea, respectively. The cases of lordosis and kyphosis recorded in specimens of *Liza* (= *Planiliza*) *abu* and *M. cephalus* were similar in severity to those in specimens "B" and "D" described in the present study. Innal et al. (2019) have recently described two cases of kyphosis in *B. pergamonensis* collected from the Dalaman River, but after examining the x-rays provided by the first author (LAJ), these cases turned out to be two cases of consecutive repetition of lordosis and kyphosis rather than kyphosis. Interestingly, this is the same locality from which the specimens of *B. xanthos* were collected.

This comparison may indicate two aspects: 1) environmental conditions in different localities have deteriorated to the same level so that they affect the growth of resident fish species; 2) varying degrees of susceptibility of the fish species to harsh environmental conditions. This is evidenced by the number of severe cases of consecutive repetition of lordosis and kyphosis in the four specimens examined in the present study.

Innal et al. (2019) related the two abnormal cases they described to different types of pollutants present in the environment and concluded that the cases of anomalies they found could be related to the level of environmental pollution present in the Dalaman River from where they obtained their fish specimens. In their study, Innal et al. (2019) found that the levels of 10 out of 14 non-metallic parameters and 11 out of 26 dissolved metals were elevated. Furthermore, their results revealed that 17 out of 156 pharmaceuticals such as escitalopram, citalopram, DEET, ephedrine, caffeine, metformin, carbamazepine, etodolac, pseudoephedrine, lidocaine, diltiazem, amisulpride, verapamil, pheniramine, diphenhydramine, venlafaxine and metoprolol were detected with elevated values. Moreover, Innal et al. (2019) found that water of the Dalaman River contains high levels of 12 pesticides out of 144 pesticides they analyzed. Considering the pollution level in the Dalaman River presented by Innal et al. (2019), we propose that pollution from different sources is the main causative agent behind the skeletal abnormalities described in the specimens of *B. xanthos* collected from the same river from which Innal et al. (2019) obtained their samples (i.e. the Dalaman River).

Although the number of studies on the health of wild and cultured fish in Turkey has increased in recent years, studies on skeletal deformities in fish such as cultured sharpsnout seabream *Diplodus puntazzo* (Yıldırım et al. 2014) and silverside *A. boyeri* (Jawad et al. 2017b) are still limited. The present study, describing different skeletal anomalies in the *B. xanthos* population from the Dalaman River, is believed to be the first study to describe such abnormalities in a wild population of this fish species.

The presence of various skeletal deformities can have a negative impact on fisheries because they can reduce the fish biomass and further reduce the value per kg of catch. Consequently, more attempts, such as research on fisheries management, should be made to determine the multiple etiological causes of these anomalies.

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