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SKELETAL ABNORMALITIES IN FOUR FISH SPECIES COLLECTED FROM THE SEA OF MARMARA, TURKEY

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ABSTRACT

Skeletal anomalies such as ankylosis (fusion of vertebrae), lordosis (ventral curvature), kyphosis (dorsal curvature) and pugheadedness have been studied in many fish species, both cultured and wild populations. Specimens of Trachurus trachurus *(Linnaeus, 1758),* Mullus surmuletus *Linnaeus 1758,* Serranus hepatus *(Linnaeus, 1758) and* Merluccius merluccius *(Linnaeus, 1758) were collected from the Sea of Marmara (Turkey) and used in this study. Deformities of the vertebral column were located in both thoracic and caudal vertebrae. In the cases of pugheadedness two different levels of severity were observed: secondary level (slight deformation) and tertiary level (severe deformation). The* S. hepatus *specimen displayed the severest level of deformity among the cases studied, ankylosis as well as pugheadedness. All cases were non-fatal as they occurred in adult individuals.*

Key words: Vertebral deformity, ankylosis, lordosis, kyphosis, pollution, environment

ANOMALIE SCHELETRICHE IN QUATTRO SPECIE DI PESCI DEL MARE DI MARMARA, TURCHIA

SINTESI

Anomalie scheletriche come l'anchilosi (fusione di vertebre), la lordosi (curvatura ventrale), la cifosi (curvatura dorsale) e l'ipoplasia della fronte sono state studiate in molte specie di pesci, sia in popolazioni di allevamento che selvatiche. Esemplari di Trachurus trachurus *(Linnaeus, 1758),* Mullus surmuletus *Linnaeus 1758,* Serranus hepatus *(Linnaeus, 1758) e* Merluccius merluccius *(Linnaeus, 1758) sono stati catturati nel Mar di Marmara (Turchia) e utilizzati in questo studio. Le deformazioni della colonna vertebrale erano localizzate in entrambe le vertebre toraciche e caudali. Nei casi di ipoplasia della fronte sono stati osservati due diversi livelli di gravità: livello secondario (deformazione leggera) e livello terziario (deformazione grave). L'esemplare di* S. hepatus *ha mostrato il livello più grave di deformità tra i casi studiati, l'anchilosi così come l'ipoplasia della fronte. Tutti i casi non sono stati fatali in quanto si sono verificati in individui adulti.*

Parole chiave: deformità vertebrale, anchilosi, lordosi, cifosi, inquinamento, ambiente

INTRODUCTION

Fish specimens with morphological deformities are fairly rare but raise concern when encountered, mainly amongst aquaculturists (Buckland, 1863), fishermen, and anglers (Fjelldal *et al.*, 2015; Näslund & Jawad, 2021) as well as naturalists and scientists, who often collected them in both private and official collections, either out of interest or for scientific examination (Hickey *et al.*, 1977; Heron *et al.*, 1988). Abnormalities should be identified as they can be important indicators of pollution or other adverse environmental factors (Klumpp *et al.*, 2002; Simon & Burskey, 2016). Therefore, numerous investigators have proposed that abnormalities within fish populations must be supervised as gauges of environmental health in aquatic ecosystems (Lemly, 1997; Sfakianakis *et al.*, 2015; Jawad & Ibrahim, 2018).

Among the fish skeletal anomalies often seen and described in several fish groups are ankylosis, lordosis, kyphosis, and pugheadedness. These deformities can be mild or severe both in aquaculture facilities and in the wild (Jawad & Ibrahim, 2018; Näslund & Jawad, 2021). Ankylosis (fusion of vertebrae) can cause deformation of the vertebral bodies either in the form of compression or combination of compression and fusion (Witten *et al.*, 2006). Lordosis is perhaps the most well described axis deformity in fishes. It can cause distress in every region of the vertebral axis. It can occur as a pre-haemal lordosis, which has been correlated considerably to the non-inflation of the swim bladder (Chatain, 1994), haemal lordosis, which is a common deformity in fishes (Jawad *et al.*, 2014; Fjelldal *et al.*, 2009), or cranial (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); like lordosis, it can be found in pre-haemal and haemal positions (Boglione *et al.*, 1995). Pugheadedness is a noticeable craniofacial skeletal anomaly in fish. It has been known as *brachygnathia superior*, but also referred to as simocephaly, snub-nose, pug-nose, lion-head, bulldog-head, or dolphin-head (Gudger, 1936). Owing to its conspicuity it has drawn specific scientific attention over several centuries (Näslund & Jawad, 2021). Pugheadedness is a brachycephalic anomaly categorised by antero-posterior compression, or hypoplasia, of the forehead. Representative pugheaded fish have brusquely rounded and short foreheads which arch sharply downward just anterior of the eyes, while the lower jaw typically remains normal-like (Branson & Turnbull, 2008; Boglione *et al.*, 2013). Several levels of severity of pugheadedness have been defined and specimens displaying the respective deformities reported (Hickey *et al.*, 1977; Lemly, 1993; Bueno *et al.*, 2015).

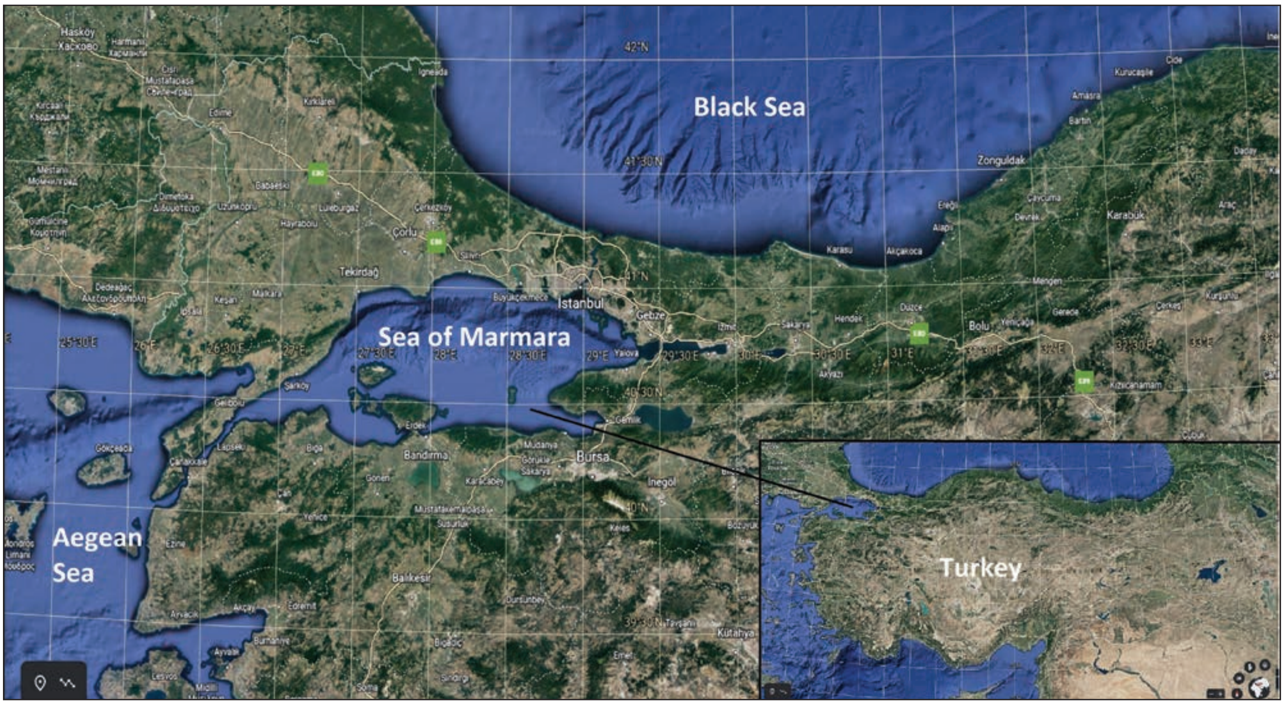

Fig. 1: Map of the study area.
Sl. 1: Zemljevid obravnavanega območja.

The Atlantic horse mackerel, *Trachurus trachurus* (Linnaeus, 1758) is a pelagic-neritic species (FAO 2010-2013), while the surmullet, *Mullus surmuletus* Linnaeus 1758, the brown comber, *Serranus hepatus* (Linnaeus, 1758) and the European hake, *Merluccius merluccius* (Linnaeus, 1758) are demersal species (Mytilineou *et al*., 2005; Smith, 1981; Muus & Nielsen, 1999). Unlike the brown comber, *S. hepatus,* which has a lower economic value*, T. trachurus*, *M. surmuletus* and *M. merluccius* are all fish of high commercial value. Even in this perspective alone, the health status of these species should be controlled and managed regularly.

For the region of Turkey and the Aegean Sea, cases of lordosis-kyphosis were reported in *M. barbatus* (Jawad et al., 2018b) and pugheadedness in *M. merluccius* (Jawad et al., 2018a). There were no records of ankylosis, lordosis-kyphosis, or pugheadedness in *T. trachurus* and *S. hepatus*. Therefore, the present study is the first to describe a case of ankylosis in a specimen of *S. hepatus*, cases of lordosis-kyphosis in *M. merluccius*, *T. trachurus,* and *M. surmuletus*, and pugheadedness in *M. merluccius*, *T. trachurus,* and *S. hepatus* collected from the Sea of Marmara, Turkey.

MATERIAL AND METHODS

Specimens of *M. merluccius* (3, 188, 243 mm TL), *T. trachurus* (6, 98–160 mm TL), *M. surmuletus* (1, 133 mm TL) *and S. hepatus* (1, 73 mm TL) were collected from different locations (40.37 N 28.13 E, 40.55 N 28.34 E, 40.30 N 28.10 E, and 40.37 N 28.13 E) in the Sea of Marmara in the period 2017–2018 (Fig. 1). All fish specimens were captured by a small commercial trawler operating at depths ranging between 50 and 180 m. In describing the vertebral column of the specimens, all vertebrae lacking haemal spines were called "abdominal vertebrae" and those presenting haemal spines were called "caudal vertebrae." These specimens exhibited lordosis, kyphosis, and pugheadedness. One normal specimen of *M. merluccius* (140 mm TL), *T. trachurus* (117 mm TL), *M. surmuletus* (140 mm TL), and *S. hepatus* (83 mm TL) were obtained for comparison. Specimens' body and fins were examined carefully for malformations, deletions, and any other morphological anomalies. The specimens were fixed in 70% ethanol and deposited in the fish collection of the Department of Fisheries, Sheep Research Institute, Bandırma, Balıkesir, Turkey. The skeletons of both normal and abnormal specimens were examined using an X-ray machine available at the Veterinary Pet Clinic in Turkey. The angle of vertebral deformation was measured from the centre of the deformity, which in the present case was located in the caudal region, using a digital protractor. To assess the degree of abnormality in the anomalous individual, the height of the curvature of the spinal column (HC) was measured. This corresponded with the distance between the tangent to the apical vertebra and a straight line which passed through the base of the two vertebrae limiting the curvature. The measurements were made with a digital caliper to the nearest 0.01 mm. The depth of curvature (DC) was calculated with the following formula using the method by Louiz *et al*. (2007):

$$DC = (HC / SL) \times 100 \text{ (SL = standard length fish)}$$

To describe vertebral shape changes independently of the individual sizes, five ratios from seven vertebral measurements were calculated.

Length ratio = dorsal length of the vertebra/ventral length of vertebra
Width ratio = anterior width of the vertebra/posterior width of the vertebra
Height ratio = dorsal height of the vertebra/ventral height of the vertebra
Thickness ratio = middle line width of the vertebra/ posterior width of the vertebra
Slenderness ratio = dorsal length of the vertebra/ posterior width of the vertebra

The purposes of these five ratios are: length ratio for wedging along vertebral length; width ratio for wedging along vertebral width; height ratio for distortion of amphicoelous shape; thickness ratio for mid-

Fig. 2: A case of pugheadedness and ankylosis in the serranid species** Serranus hepatus**. A, normal specimen, 83 mm TL; B, abnormal specimen, 73 mm TL.
Sl. 2: Primer popačenosti glave in ankiloze pri volčiču (**Serranus hepatus**). A - normalen primerek, 83 mm telesne dolžine; B – primerek z anomalijami, 73 mm telesne dolžine.

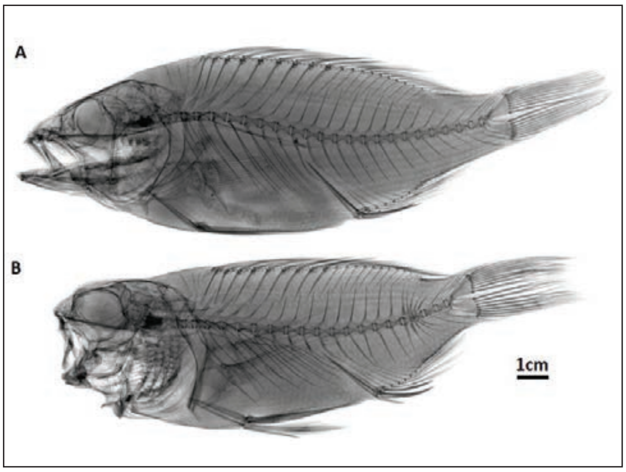

Fig. 3: Radiograph of *Serranus hepatus****. A - normal specimen, 83 mm TL, B - abnormal specimen, 73 mm TL, exhibiting pugheadedness and ankylosis.***
Sl. 3: Radiografija primerka vrste *Serranus hepatus****. A - normalen primerek, 83 mm telesne dolžine; B – primerek s popačeno glavo in ankilozo, 73 mm telesne dolžine.***

centrum thickness; and slenderness ratio for ventral slenderness. All measurements were made by the same person and using the same instrument in order to increase the accuracy of the measurements and reduce variability owing to measurement error.

RESULTS

The description of the cases of lordosis-kyphosis in *M. merluccius, T. trachurus* and *M. surmuletus*, and pugheadedness in *M. merluccius, T. trachurus* and *S. hepatus* is provided below.

Ankylosis
Family: Serranidae
Serranus hepatus (Figs. 2 and 3)

This specimen had two deformities, ankylosis at the posterior caudal vertebrae and pugheadedness. Compared externally to the normal specimen of a nearly equal size, the abnormal specimen exhibited a short and stubby caudal peduncle area and a pug-head deformity. Pectoral and caudal fins were normal. The lateral line appeared normal. Radiographs showed an incidence of ankylosis in five vertebrae of the posterior part of the caudal region. Caudal vertebrae 7–11 were preserved, the anterior half of the 11th vertebra was lost, the whole centra of vertebrae 8–10 were lost, coalescence of the neural and haemal spines of these vertebrae were preserved, and the anterior half of 11th vertebra was lost. The remaining vertebrae of the vertebral column were normal in shape, but they were directed upwards.

Fig. 4: *Trachurus trachurus****. A - abnormal specimen, 243 mm TL; B - normal specimen, 117 mm TL.***
Sl. 4: *Trachurus trachurus****. A - primerek z anomalijami, 243 mm telesne dolžine; B - normalen primerek, 117 mm telesne dolžine.***

The haemal spine of the 3rd caudal vertebra was directed backwards toward the haemal spine of the 4th caudal vertebra instead of downwards.

Kyphosis
Family: Carangidae
Trachurus trachurus (Figs. 4 and 5)

The hump in the anterior part of the vertebral column was the only externally visible physical anomaly (Fig. 4). Compared to the radiograph of the normal specimen, the radiograph of the deformed specimen showed upward arching of thoracic vertebrae (V1-V7), while the descending part of the vertebral column is formed of vertebrae 8–11 (Fig. 5).

The curvature in the vertebral column of the deformed specimen seemed to affect the dimensions of the thoracic vertebrae involved in the arching. Vertebrae 3–6 showed an increased height on the ventral side (0.019–0.021) and reduced height on the dorsal side (1.200–1.201). Vertebrae 1–3 were slightly wedged (1.101–1.110) (having a reduced ventral length compared to the dorsal length). Vertebrae 5–11 had reduced midline widths (0.021–0.025). The amphicoelous centra of vertebrae 2 and 3 were distorted such that the height was increased on the dorsal side (0.001–0.002). Slenderness and thickness were reduced in vertebrae 2–3 (0.001–0.003).

Consecutive repetition of lordosis-kyphosis
Family: Merlucciidae
Merluccius merluccius (Fig. 6)

A radiograph of the deformed specimen showed two lordotic and two kyphotic regions extending along all vertebrae from V1 to V50. Each region involved multiple vertebrae. The vertebrae forming the 1st lordotic arch were V–V12, the 1st kyphotic

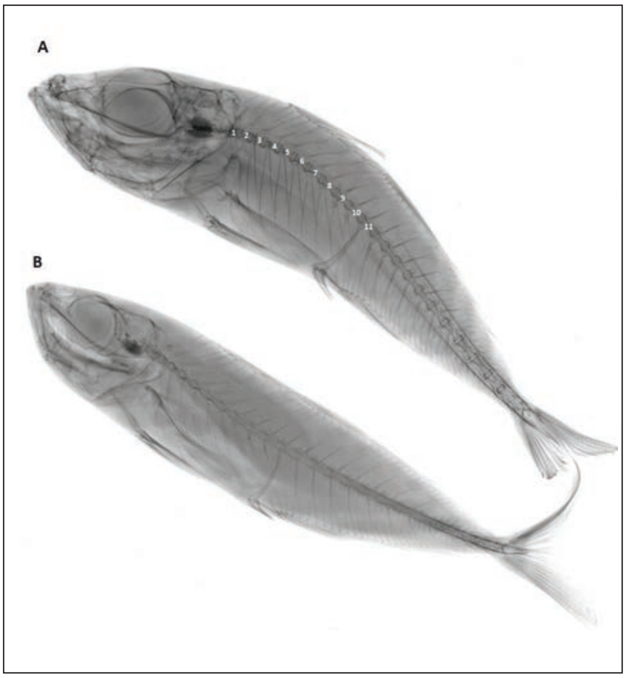

Fig. 5: Radiograph of** Trachurus trachurus**. A - abnormal specimen exhibiting kyphosis, 160 mm TL. The vertebrae involved in the incidence of abnormality are numbered 1–11; B - normal specimen, 117 mm TL.
Sl. 5: Radiografija primerka vrste** Trachurus trachurus**. A - primerek s kifozo, 160 mm telesne dolžine. Vretenca, pri katerih je anomalija izražena, so oštevilčena od 1 do 11; B - normalen primerek, 117 mm telesne dolžine.

arch contained vertebrae 4–20, the 2nd lordotic arch comprised vertebrae 12–29, and in the 2nd kyphotic arch involved vertebrae 20–51 (Fig. 6). The value of the lordotic angle "A" was 146.7°, the kyphotic angle "B" was 152.37°, the lordotic angle "C" was 160.22° and the kyphotic angle "D" was 156.03°. In general, the dimensions of the vertebrae involved in lordosis-kyphosis repetition were not affected. The amplitude of the curvatures of the angles A, B, C, and D were 34.5, 23, 16.1 and 11.5 mm, respectively.

Family: Carangidae
Trachurus trachurus (Figs. 7–8)

There were 4 specimens of *T. trachurus* displaying consecutive recurrence of lordosis-kyphosis, as shown in Figures 7a-d. The spinal deformities in the deformed specimens were visible externally, already upon capture. The severity of the anomalies in the affected specimens ranged from mild in specimen "A," medium in specimens "B" and "C," to severe in specimen "D." No other deformities were detected on the bodies of the deformed fish.

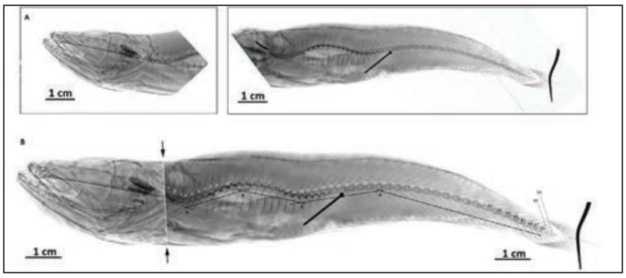

Fig. 6: Radiograph of a** Merluccius merluccius **specimen, 139 mm TL, exhibiting consecutive repetition of lordosis-kyphosis. A - general view of the deformed vertebral column; B - vertebrae (numbered) involved in the incidence of repetition of lordosis-kyphosis.
Sl. 6: Radiografija primerka vrste** Merluccius merluccius**, 139 mm telesne dolžine, s ponavljajočima se lordozo in kifozo. A - pogled na deformirano hrbtenico; B - vretenca (oštevilčena), pri katerih je anomalija izražena.

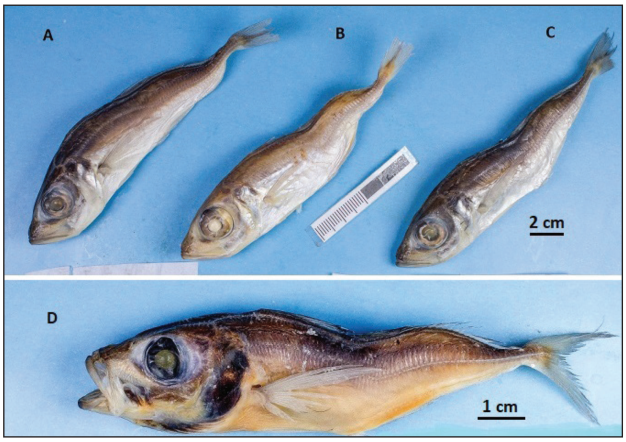

Fig. 7: Specimens (A–D) of** Trachurus trachurus **of different sizes displaying different levels of consecutive repetition of lordosis-kyphosis.
Sl. 7: Različno veliki primerki (A–D) šnjura (**Trachurus trachurus**) z izraženim ponavljanjem lordoze-kifoze.

Radiographs of the four anomalous specimens revealed various recurrence of lordosis and kyphosis. Specimen "A" exhibited two lordotic and two kyphotic arches, specimens "B" and "C" exhibited two lordotic arches and one kyphotic arch, and specimen "D" exhibited one lordotic and one kyphotic arch.

In specimen "A," the vertebrae involved in the 1st kyphotic arch were V1–V9, with V5 at the top centre of the arch. The 1st lordotic arch involved V6–V15, with V10 at the bottom centre of the curve. The 2nd kyphotic arch included V11–V18, with V14 located at the top centre of the arch. The 2nd lordotic arch involved V16–V22, with V19 at the bottom centre of the curve.

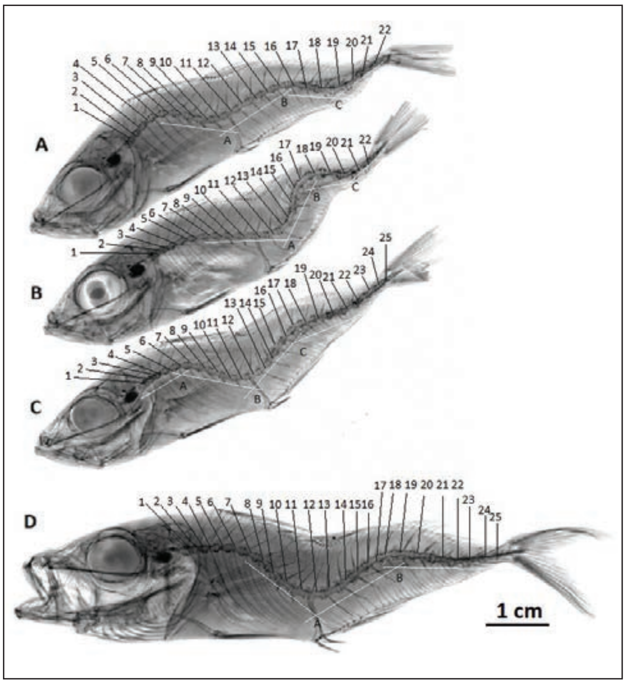

Fig. 8: Radiographs of four specimens (A–D) of** Trachurus trachurus **displaying different levels of consecutive repetition of lordosis-kyphosis. The vertebrae involved in the incidences of abnormality are numbered.
Sl. 8: Radiografije štirih primerkov šnjura (**Trachurus trachurus**) (A–D) izraženim ponavljanjem lordoze-kifoze na različnih nivojih. Vretenca, pri katerih je anomalija izražena, so oštevilčena.

In specimen "B," V7–V16 were included in the 1st lordotic arch, with V12 at the bottom centre of the arch. The 1st kyphotic arch included V13–V20, with V17 located at the top centre of the arch. The 2nd lordotic arch included V18–V22, with V20 at the bottom centre of the arch.

In specimen "C," the 1st kyphotic arch contained vertebrae 1–11, with V6 at the top centre of the arch. The 1st lordotic arch involved V7–V16, with V12 found at the bottom centre of the arch. The 2nd kyphotic arch included V13–V21, with V 17 at the top centre of the arch.

In specimen "D," the lordotic arch involved V7–V18, with V12 situated at the bottom centre of the arch. The kyphotic arch included V13–V22, with V19 positioned at the top centre of the arch.

The values of angles "A," "B" and "C" in specimen "A" were 139°, 138° and 140°, respectively. The values of angles "A," "B" and "C" in specimen "B" were 118.8°, 125.3° and 150°, respectively. The values of angles "A," "B," and "C" in specimen "C" were 140°, 121.5° and 156.2°, respectively. The values of angles "A" and "B" in specimen "D" were 110.8° and 149.7°, respectively.

Fig. 9: Two specimens of** Mullus surmuletus**. A - normal, 140 mm TL; B - abnormal specimen, 133 mm TL, exhibiting consecutive repetition of lordosis-kyphosis.
Sl. 9: Dva primerka progastih bradačev (**Mullus surmuletus**). A - normalen primerek, 140 mm telesne dolžine; B - primerek z izraženim ponavljanjem lordoze-kifoze s telesno dolžino 133 mm.

The depth of the curvatures of the angles "A, B, C" in specimen "A" were 26, 20, 15, and 14 mm, respectively. The depth of the curvatures of the vertebral column A, B, C in specimen "B" were 27, 30, and 15 mm, respectively. The depths of the curvatures of the vertebral column A, B, C in specimen "C" were 26, 27, and 15 mm, respectively. The depths of the curvatures of the angles A and B in specimen "D" were 42 and 21 mm respectively.

The kyphosis-affected vertebrae of the four specimens of *Trachurus trachurus* collected from the Sea of Marmara, Turkey, are shown in Table 1.

Family: Mullidae
Mullus surmuletus (Figs. 9 and 10)

One specimen exhibited consecutive repetition of lordosis-kyphosis. Externally, the fish appeared to have a wide and high hump extending from the anterior to the posterior edges of the dorsal fin. Posteriorly to the soft rays of the dorsal fin, the dorsal edge of the body displayed a notch followed by a lower and shorter hump over the caudal peduncle region. Lateral line scales were disturbed starting from below the notch, and posteriorly directed toward the caudal fin. No other abnormalities were observed.

Compared to that of a normal specimen, the radiograph of the anomalous specimen revealed two lordotic and two kyphotic arches. The 1st lordotic arch involved V6–V14, with V10 at the bottom centre of the arch. The 1st kyphotic arch included V12–17, with V15 at the top of the arch. The 2nd lordotic arch encompassed V15–V19, with V18 at

Tab. 1: The various effects of kyphosis on vertebrae of the four deformed specimens of *Trachurus trachurus* ***collected from the Sea of Marmara, Turkey. V = vertebra.***
Tab. 1: Različni učinki kifoze na vretencih štirih deformiranih primerkov šnjurov *Trachurus trachurus* ***iz Marmarskega morja (Turčija). V = vretenca.***

Specimen	Effect	Site of the vertebrae	Vertebrae affected	Value (mm)
A	Increased height	ventral side	V4, 5, 6, 16 and 19	0.020 – 0.023, 0.021 – 0.022, 0.020 – 0.021, 0.021 – 0.024
B	Increased height	ventral side	V18 and V19	0.022 – 0.023, 0.020 – 0.022
C	Increased height	ventral side	V16 and V17	0.024 – 0.026, 0.019 – 0.021
D	Increased height	ventral side	V18 and V19	0.025 – 0.026, 0.022 – 0.024
A	Reduction	dorsal side	V4, 5, 6, 16 and 19	0.210 – 0.230, 0.221 – 0.224, 0.210 – 0.214, 0.221 – 0.231
B	Reduction	dorsal side	V18 and 19	0.213 – 0.223, 0.214 – 0.216
C	Reduction	dorsal side	V16 and V17	0.217 – 0.221, 0.217 – 0.225
D	Reduction	dorsal side	V18 and 19	0.227 – 0.232, 0.221 – 0.229
A	Wedged	-	V4, 5, 19 and 20	0.345 – 0.365, 0.335 – 0.338, 0.332 – 0.339,0.331 – 0.335
B	Wedged	-	V14 and V15	0.311 – 0.318,0.324 – 0.327
C	Wedged	-	V14, V15, and V20	0.311 – 0.314,0.321 – 0.325
D	Wedged	-	V8 and V14	0.331 – 0.338,0.311 – 0.317
A	Reduced midline	-	V17	0.023–0.027
C	Reduced midline	-	V17	0.024 – 0.28
D	Reduced midline	-	V5, 6, and 7	0.019–0.021,0.021–0.022, 0.025–0.028
A	Less Slenderness & thickness	-	V2 – V10	0.002 – 0.003
B	Less Slenderness & thickness	-	V5 – V12	0.001 – 0.004
C	Less Slenderness & thickness	-	V14 – V19	0.002 – 0.005
D	Less Slenderness & thickness	-	V16 – V23	0.001 – 0.002

the bottom centre of the arch. The 2nd kyphotic arch contained V18–V24, with V21 at the top centre of the arch.

The depths of the curvatures of the angles A, B, C, and D were 4.5, 6.5, 6, and 6.5 mm, respectively. The values of the angles A–D were 100°, 102°, 98°, and 120°, respectively.

The different vertebral calculated ratios appeared to be affected by the position of the vertebra and the curvature of the vertebral column. Vertebrae 8, 9, 14, 15, and 20 showed an increased height on the ventral side (0.022–0.024) and reduced on the dorsal side (0.230–0.236). Vertebrae 14, 15, and 21 were wedged (0.045–0.055) (having a reduced ventral length relative to their dorsal length). Vertebrae 3–6 had reduced midline widths (0.023–0.028).

The centra of the vertebrae 9–13 were compressed and slightly deformed, and their neural spines were displaced.

Tab. 2: Morphometric measurements (mm) of the normal and abnormal specimens of *Serranus hepatus, Trachurus trachurus**, and*** *Merluccius merluccius* ***collected from the the Sea of Marmara Sea, Turkey.***
Tab. 2: Morfometrične meritve (mm) normalnih primerkov in primerkov z anomalijami vrst *Serranus hepatus, Trachurus trachurus* ***in*** *Merluccius merluccius**, ujetih v Marmarskem morju (Turčija).***

Species	Status of the specimen	Total length	Standard length	Head length	Preorbital length	Eye diameter	Postorbital length
Serranus hepatus	Normal	83	70.7	28.6	7.1	7.9	11.4
	Abnormal	73.2	57.1	24.3	2.5	7.8	11.3
Trachurus trachurus	Normal	122	98.3	26.7	15.6	15.8	16.2
	Abnormal	193.8	265.6	57.5	12.7	14.4	15.6
Merluccius merluccius	Normal	225.0	192.7	63.6	20.0	13.6	32.9
	Abnormal	281.8	251.8	79.1	15.5	12.7	31.7

Pugheadedness
Family: Serranidae
Serranus hepatus (Figs. 2 and 3)
Family Carangidae
Trachurus trachurus (Figs. 11 and 12)
Family: Merlucciidae
Merluccius merluccius (Figs. 13 - 15)

The abnormal pugheaded specimens of the species *S. hepatus*, *T. trachurus, and M. merluccius* (Figures 2b, 12, and 14a) were compared to respective normal specimens (Figs. 2a, 4b, and 14b). The morphometric measurements of the head in both normal and abnormal specimens are shown in Table 2.

The three specimens that exhibited pugheadedness appeared to have a short neurocranium and upper jaw, and an abnormal (reduced) lower jaw. The snout appeared nearly absent in *S. hepatus*, and moderately present in *T. trachurus* and *M. merluccius*. The mouth in *M. merluccius* was virtually closed by the dropped anterior part of the skull that left only a small opening, but open in *S. hepatus* and *T. trachurus*. In the case of *S. hepatus*, the shortening of the snout had brought the steep forehead close to the eye and moved the anterior nostril ventrally towards the mouth. Most evidently in the case of *S. hepatus*, but to a certain degree also in the specimens of the other two species examined, the head was ball-shaped and tilted upward, resulting in a conspicuous curved area immediately behind it. As a result of the head displacement, the mouth was turned slightly upward in the case of *M. merluccius*, but not in the other two specimens studied.

Compared to normal specimens, the radiographs of the anomalous pugheaded specimens revealed the following deformities: a complete absence of bones of the anterior part of the skull in *S. hepatus*; short vomer, parasphenoid, and maxillaries, displacement and/or curvature of the nasals, frontals, vomer, and palatines in *T. trachurus* and *M. merluccius*; deformed upper jaw teeth in *M. merluccius*. For these reasons, the forehead was upraised and steep in the pugheaded specimen, which in turn exerted pressure on abdominal vertebrae 1-3 making them curve slightly downward (instead of running in a straight line) and stand closer to each other. In the specimens of *T. trachurus* and *M. merluccius*, the otoliths were displaced backwards and appeared to be shorter in length compared to those of normal specimens. In addition, the ventral ends of the three supraneurals located just behind the skull in *S. hepatus* appeared close packed rather than set at equal distances as in the normal specimen.

DISCUSSION

This is the first investigation examining the occurrence and type of vertebral anomalies in the inspected adult wild teleost fish species from the Sea of Marmara, Turkey. The goal was to identify skeletal anomalies and determine a possible relationship between these abnormalities and environmental factors. In the present study, no water analyses were carried out as it is not in the scope of the project that this study is sitting in.

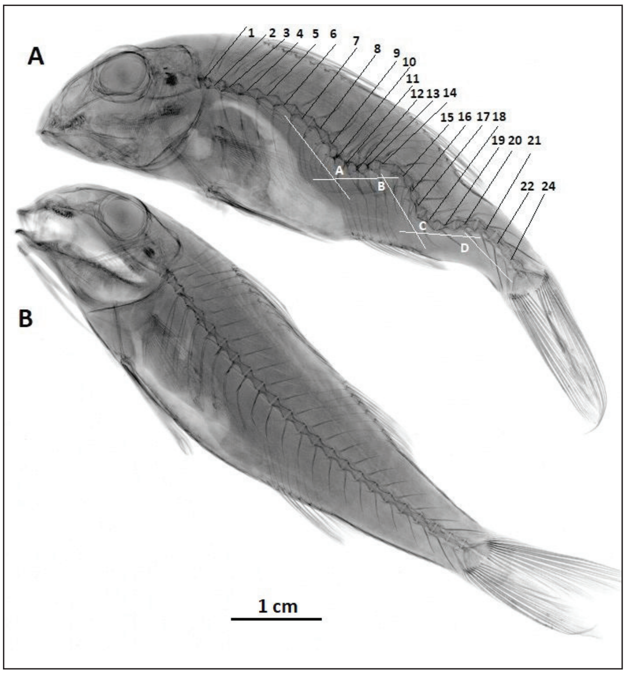

Fig. 10: Radiograph of two specimens of* Mullus surmuletus*. A - abnormal specimen, 133 mm TL, exhibiting consecutive repetition of lordosis-kyphosis. The vertebrae involved in the incidences of abnormality are numbered; B - normal specimen, 140 mm TL.
Sl. 10: Radiografija dveh primerkov vrste* Mullus surmuletus*. A - primerek s telesno dolžino 133 mm TL z izraženim ponavljanjem lordoze-kifoze. Vretenca, pri katerih je anomalija izražena, so oštevilčena; B - normalen primerek, 140 mm telesne dolžine.

Fig. 11: Specimen of* Trachurus trachurus*, 243 mm TL, exhibiting pugheadedness.
Sl. 11: Primerek šnjura s popačeno glavo, 243 mm telesne dolžine.

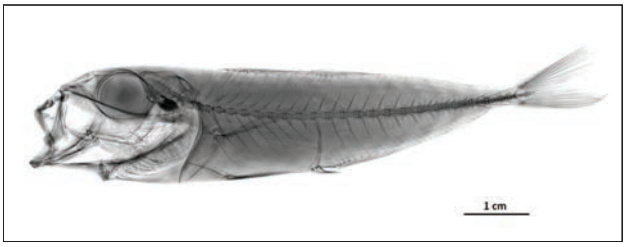

Fig. 12: Radiograph of specimen of* Trachurus trachurus*, 243 mm TL, exhibiting pugheadedness.
Sl. 12: Radiografija primerka šnjura s popačeno glavo, 243 mm telesne dolžine.

There is a considerable number of works on wild fish abnormalities (Divanach *et al.*, 1997; Jawad *et al.*, 2013; Jawad & Liu, 2015) related to deformities. Investigators have studied both genetic (Ishikawa, 1990) and epigenetic issues as conceivable causes of such anomalies (Boglione *et al.*, 1995), as well as environmental features such as temperature, light, salinity, pH, low oxygen concentrations, inadequate hydrodynamic conditions.

In this study, the anomalous specimens presented cases of ankylosis (*S. hepatus*), kyphosis (*T. trachurus*), kyphosis-lordosis (*M. merluccius*, *T. trachurus*, and *S. surmuletus*), and pugheadedness (*S. hepatus*, *T. trachurus*, and *M. merluccius*).

Ytteborg *et al.* (2012) proposes 4 characterising stages of vertebral fusion that may result in spinal fusion (as in the specimen of *S. hepatus*): (i) The early stages in the fusion process are characterised by disordered and proliferating osteoblasts and chordoblasts. (ii) Then, these proliferating cells go through a metaplastic shift: proliferating osteoblasts co-express a mixed signal of both chondrogenic and osteogenic markers and proliferating chordoblasts change transcription to a more osteogenic profile. (iii) As the pathology proceeds, the elastic membrane adjoining the notochord becomes fragmented and the notochordal sheath loses is integrity. (iv) Finally, mineralisation of intervertebral regions and arch centra can be seen.

Evidence from several mammalian investigations has suggested that deviations in the balance between cell death and cell propagation may cause malformations (Kanda & Miura, 2004). The results of the studies of Ytteborg *et al.* (2012) suggest that an augmented development of osteoblasts in progress zones is partially steadied by increased cell death, subsequently, the phase of metaplastic shift to vertebral fusion takes place, followed by a phase of notochordal sheath vitiation, where this sheath was present in a reinstated shape after brief deformation (Yu *et al.*, 2005); consequently, a tear in this sheath might lead to a spinal abnormality.

It is possible that the anomalous specimen of *S. hepatus* was confronted by adverse environmental influences that could give rise to such type of vertebral deformity. Since the specimen reached a sub-adult stage, the anomaly was not deadly; however, it would have definitely affected its mobility in some way when the fish reached adulthood. Except for the distorted caudal peduncle region, the dorsal and anal fins and the remaining fins were found in an apparently perfect state.

Fig. 13: *Merluccius merluccius*. ***A - abnormal specimen, 240 mm TL, exhibiting pugheadedness; B - normal specimen, 188 mm TL.***
Sl. 13: *Merluccius merluccius*. ***A - primerek s popačeno glavo, 240 mm telesne dolžine; B - normalen primerek, 188 mm telesne dolžine.***

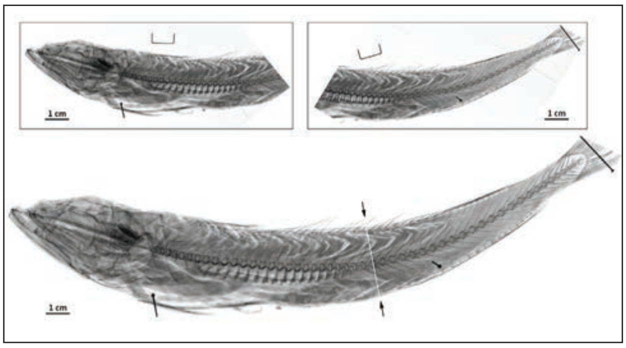

Fig. 14: Radiograph of two specimens of *Merluccius merluccius*, ***139 and 188 mm TL, respectively, exhibiting pugheadedness.***
Sl. 14: Radiografija dveh primerkov vrste *Merluccius merluccius*, ***s telesnima dolžinama 139 in 188 mm, z izraženo popačenostjo glave.***

The variations in the shape of the vertebral column in the incidences of lordosis and kyphosis in the specimens of *M. merluccius*, *T. trachurus* and *S. surmuletus* were linked to the anterior-posterior (i.e., cranial-caudal) compression along the spine. Radiographs of the deformed specimens (Figs. 5, 6, 9, and 11) revealed structural deformations; the normal amphicoelous (hourglass) shape of the vertebrae was distorted, with the vertebral height reduced on the convex and increased on the concave side of the vertebral column. In addition, vertebrae at the approximate bottom centre of the curvature (in the case of a lordotic arch) were wedged, with the length on the concave side of the vertebral column reduced compared to the convex length of the vertebral column. Also, the midline width was significantly lower in some vertebrae. Similar variations were detected in *Poecilia reticulata* by Gorman *et al*. (2010). They suggested that the perceived deviations in vertebral bone structure could be a result of either (1) distortion of normal vertebral shape or (2) active remodelling of vertebral osteoid bone as a consequence of extrinsic forces. They added that vertebral growth in fishes is dissimilar from that of other animal models. The *Poecilia reticulata* which they investigated had vertebrae comprised of acellular bone (i.e., missing entrenched osteocytes and constructed by intramembranous ossification) (reviewed in Witten and Huysseune 2009). Consequently, further investigations of vertebral wedging in *M. merluccius*, *T. trachurus* and *S. surmuletus* as well as other fish species exhibiting lordosis and kyphosis in the future should test cellular activity at the intervertebral region, the presumed growth zone of guppy vertebrae (Inohaya *et al*., 2007), to establish whether there is variation of growth in curved individuals.

The cases of lordosis, kyphosis and consecutive recurrence of lordosis-kyphosis examined in the present study were compared with similar cases in other fish species collected from the Turkish waters. Jawad & Öktener (2007) studied these abnormalities in *Liza* (= *Planiliza*) *abu* collected from Lake Ataturk Dam. Jawad *et al*. (2017a, c) and Jawad *et al*. (2018b) described cases of lordosis, kyphosis, and consecutive repetition of lordosis-kyphosis in the *Atherina boyeri* collected from the Homa Lagoon, Izmir, and in the *Mullus barbatus* and *Mugil cephalus* collected from the northern Aegean Sea, respectively. The case of lordosis and kyphosis seen in the specimens of *Liza* (= *Planiliza*) *abu* and *M. cephalus* were similar in severity to that of specimen "B" of *T. trachurus* described in the present study. The case of *A. boyeri* (Jawad *et al*., 2017c) is similar in severity to that of *T. trachurus* specimen "C." The specimen of *M. barbatus* with consecutive repetition of lordosis-kyphosis described be Jawad *et al*. (2018b) is similar in severity to that of *T. trachurus* specimen "A" described in the present study.

Several authors have shown that bone modelling may be affected by water with reduced oxygen levels through its influence on bone mineral composition (Martens *et al*., 2006). In the waters of the Sea of Marmara in general, the variation in temperature during the years suggests comparable disparity in oxygen levels, with extremely low levels in summer, when the temperature and salinity are at their highest (Keskin *et al*., 2011; Becker *et al*., 2015). Hypoxia, a deficiency of oxygen, is a recognised cause of teratogenic incidences in the musculoskeletal system during embryonic development and during the first larval stage. Hypoxia can also initiate cell apoptosis, a key process in these stages (Shin *et al*,. 2004). During development in fish, sub-lethal hypoxia can increase the incidence of malformations (Eva *et al*., 2004). Cases of hypoxia were reported from different parts of the Sea of Marmara (Yüksek, 2016; Yalçın *et al.*, 2017). Any deformity in the shape of the vertebrae will have a direct effect on the swimming capability of the fish and its survival (Koumoundouros *et al.*, 1997), and there a noteworthy correlation between the severity of lordosis and swimming performance has been confirmed in sea bass (*Dicentrarchus labrax*), at least in juveniles (Peruzzi *et al.*, 2007).

In teleostean fishes, the severity of pugheadedness can be categorised in three ~~four~~ levels: primary, secondary, and tertiary (Hickey *et al.*, 1977). The case of *S. hepatus* represented tertiary-stage pughead anomaly as per the ranking system defined by Hickey *et al.* (1977). The mouth in this specimen was wide open in contrast to the severe cases observed in other fish species, *Johnius aneus* (Al-Hassan & Na'amma, 1988) and *Pagrus auratus* (Jawad and Hosie 2007), in which the mouth was nearly closed. The specimens of *T. trachurus* and *M. merluccius,* exhibited secondary level pugheadedness according to the system of Hickey *et al.* (1977).

Based on an examination of the mouth it was concluded that due to incomplete closure of the mouth the *S. hepatus* specimen could have lost the ability to feed on the food it usually consumed. In contrast, the pugheaded specimens of *T. trachurus* and *M. merluccius* could have been limited in their choice of food items due to the hindered opening of the mouth. Such deformities, in fact, may lead to the inability to contend for food and, consequently, decrease in growth rate (Bortone, 1971; Hickey, 1973). But the present pughead specimens of *T. trachurus* and *M. merluccius* showed no signs of poor health, so feeding was likely unrestricted.

The only works published on the incidence of pugheadedness in fishes from Turkey are by Jawad *et al.* (2017a) on *Nemipterus randalli*, Jawad *et al.* (2017b) on *Pagellus erythrinus* and Jawad *et al.* (2018a) on *Merluccius merluccius*. Specimens of these three species were collected from the Aegean Sea. Based on the descriptions of abnormalities, the case of *S. hepatus* in the present study was similar in severity to that of *N. randalli* described by Jawad *et al.* (2017a), while the cases of *T. trachurus* and *M. merluccius* in the present study were similar in severity to those of *P. erythrinus* and *M. merluccius* described by Jawad *et al.* (2017b) and Jawad *et al.* (2018a), respectively. These similarities may be indicative of two issues: 1) that the environment in both the Aegean Sea and the Sea of Marmara is equally degraded to the point of affecting the development of the fish species living in it. The pugheaded specimens of *M. merluccius* obtained from the Aegean Sea and the Sea of Marmara seem to corroborate that; and 2) a variable degree of vulnerability of fish species to harsh environmental factors. This is evident from the different levels of severity of pughead deformity in the three species examined in the present study, with *S. hepatus* exhibiting tertiary level and *T. trachurus* and *M. merluccius* secondary level of pugheadedness according to the system of Hickey *et al.* (1977).

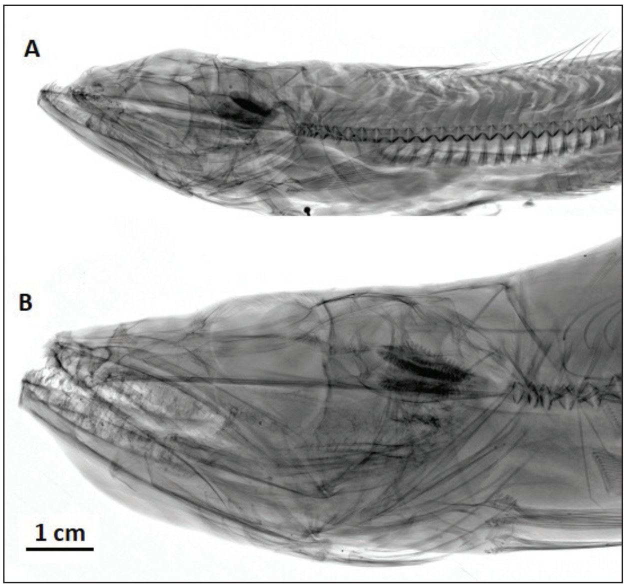

Fig. 15: Radiograph of heads of specimens of *Merluccius merluccius*. ***A - abnormal specimen, 139 mm TL, displaying pugheadedness; B - normal specimen, 188 mm TL.***
Sl. 15: Radiografija glave primerkov vrste *Merluccius merluccius*. ***A - primerek s popačeno glavo, 139 mm telesne dolžine; B - normalen primerek, 188 mm telesne dolžine.***

The cause of the manifest pughead deformity in the three specimens of *S. hepatus*, *T. trachurus,* and *M. merluccius* is unknown, but may have originated in early development (Cobcroft *et al.*, 2001). Genetic and epigenetic factors can lead to pughead anomaly (Dahlberg, 1970; Sloof, 1982). Mutations or recombination of genes are heritable but not lethal (Browder *et al.*, 1993). Contaminants such as trace metals (Nakamura, 1977; Valentine, 1975; Bengtsson, 1991) may be a likely cause as they disrupt the skeletal development by decreasing the concentrations of mobilised calcium and phosphorous within the body and hinder enzymes essential for bone metabolism (Yamashita & Hayashi, 1985; Ludwig *et al.*, 1995).

Unlike in hatcheries, the occurrence of pughead deformity in wild fish populations is infrequent. This is potentially because abnormal individuals in hatcheries are sheltered and the predation factor is nil (Bortone, 1971; Riehl & Schmitt, 1985). Consequently, the survival rate and frequency of anomalous individuals among adults there can be higher (Cobcroft *et al.*, 2001). Other investigators proposed that such abnormality is recurrent due to overcrowding (Shariff *et al.*, 1986), xenobiotics (Haga *et al.*, 2003), nutrition (Cobcroft *et al.*, 2001), inbreeding (Sadler & King, 2001) and dietary shortages (Takeuchi *et al.*, 1998).

The economy of the fish catch might be largely affected by the presence of different skeletal anomalies, as such deformities have the potential to reduce the weight of the fish and their value per kg. Consequently, more effort should put in improving the management of the fisheries industries and discovering the aetiological reasons behind the anomalies. Also, in order to assess the economic factors we should ascertain the prevalence of anomaly types in the wild.

CONCLUSIONS

Four types of skeletal abnormalities – ankylosis, lordosis, kyphosis and pugheadedness – were detected in four marine fish species collected from the Sea of Marmara, Turkey. Such anomalies were found in both abdominal and caudal regions of the vertebral column, occurring in mild and severe forms. The *S. hepatus* species appeared more vulnerable to the factors causing such anomalies than other species examined. The results of the present study can be considered as preliminary health status indicators for the environment of the Sea of Marmara and suggest that this sea environment needs to be studied further in terms of pollution in order to accurately determine its condition.

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SKELETNE ANOMALIJE PRI ŠTIRIH VRSTAH RIB IZ MARMARSKEGA MORJA (TURČIJA)

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POVZETEK

Avtorji so raziskovali skeletne anomalije kot so npr. ankiloza (otrdelost sklepov), lordoza (ventralna ukrivljenost), kifoza (dorzalna ukrivljenost) in popačenost glave na štirih vrstah morskih rib. Primerke šnjurov Trachurus trachurus *(Linnaeus, 1758), progastih bradačev* Mullus surmuletus *Linnaeus 1758, volčičev* Serranus hepatus *(Linnaeus, 1758) in osličev* Merluccius merluccius *(Linnaeus, 1758) so za potrebe pričujoče raziskave ulovili v Marmarskem morju (Turčija). Deformacije hrbtenice so ugotovili na prsnih in repnih vretencih. Primere popačenosti glave so ugotovili na dveh različnih nivojih in sicer sekundarnem nivoju (neznatna popačenost) in terciarnem nivoju (huda popačenost). Najbolj izrazite anomalije so opazili pri osebku vrste* S. hepatus, *kjer so ugotovili ankilozo in popačenost glave. Vsi ugotovljeni primeri niso bili smrtno nevarni, saj so se pojavljali pri odraslih primerkih.*

Ključne besede: deformacije vretenc, ankiloza, lordoza, kifoza, onesnaževanje morja, okolje

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