FIRST ABNORMALITIES INVESTIGATION IN *MULLUS BARBATUS* (MULLIDAE) FROM THE EASTERN COAST OF ALGERIA

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Abstract. Red mullet *Mullus barbatus*, Linnaeus, 1758, is a benthic marine fish. Because of its wide geographic distribution and close association with sediments, red mullet is commonly used as a sentinel species for pollution assessment along the Mediterranean coast. A total of 500 specimens were examined from the Gulf of Bejaia, eastern coast of Algeria, from June 2018 to June 2019 by X-ray radiography and histopathological examination. We observed for the first time in Algeria complex spinal column deformities (kyphosis, lordosis, and scoliosis), vertebral coalescence, vertebral fusion, missing of one pelvic fin, caudal fin abnormalities, congested cheek, lymphocystis, ovary malformation, and asymmetric testis. Additionally, fibrosis, inflammatory reaction, melano-macrophage centres, necrosis, atresia, and disturbance of normal tissue were detected by histopathology examination.

INTRODUCTION

The Algerian coast is under a significant anthropogenic pressure (Benkhadda et al. 2020; Mankou-Haddadi et al. 2021). Specific mining operations, several factories (agro-food), and waste dumps (metallic mercury, pharmaceuticals, pesticides, etc.) are frequently located upstream of waterways flowing into the Gulf of Bejaia (Aissioui et al. 2021).

The emergence of various deformities that may indicate the presence of ecosystem disturbances can be used to track and monitor changes in fish habitats (Gavruseva 2020). Abnormalities are irreversible, natural or anthropogenic deviations from the typical morphology of wild fish (Divanach et al. 1996). Lymphocystis disease is one of the oldest and most widespread viral diseases of fish (Borrego et al. 2017). Different types of gonad malformations such as hermaphroditism, asymmetry, atrophy, segmented, branched gonads were described in many fish populations (Hliwa et al. 2011; Rajasilta et al. 2016). Skeletal deformities (kyphosis, lordosis, scoliosis, vertebral coalescence and fusion, fin abnormalities) are a major factor that affects the production cost (Berillis 2015).

Due to its extensive geographic distribution and close association with sediments, the red mullet is considered one of the most crucial sentinel species for the Mediterranean (Porte et al. 2002; Regoli et al. 2002). *Mullus barbatus* Linnaeus, 1758 is a marine demersal fish that lives at depths of up to 300–400 m. This fish in particular is one of the most important targets of bottom-trawl fishing operations (Tserpes et al. 2019).

In Algeria, many studies have been carried out on *M. barbatus* such as parasitism (Boualleg et al. 2010; Ramdane et al. 2010), reproduction (Hebbar et al. 2012), mortality (Hebbar and Boutiba 2015), population dynamic parameters (Bensahla Talet et al. 2016), feeding habits (Boudraa et al. 2018), contamination of red mullet by heavy metal (Atoui et al. 2019; Aissioui et al. 2021). However, no studies have investigated the anatomical and histological abnormalities of this species in this region.

Fish anomalies are frequently easy to identify macroscopically and thus offer an important advantage over other methods, especially for researchers operating in the field without access to laboratory equipment (Jawad and Abed 2021).

The objectives of the present paper were to report and describe for the first time the abnormalities in *M. barbatus* from the Gulf of Bejaia. The possible causes supposed to be involved in these abnormalities were discussed.

MATERIALS AND METHODS

Sample collection and treatment

The sampling area corresponds to the commercial fisheries from the Gulf of Bejaia, in the eastern coast of Algeria (Figure 1). From June 2018 to June 2019, a
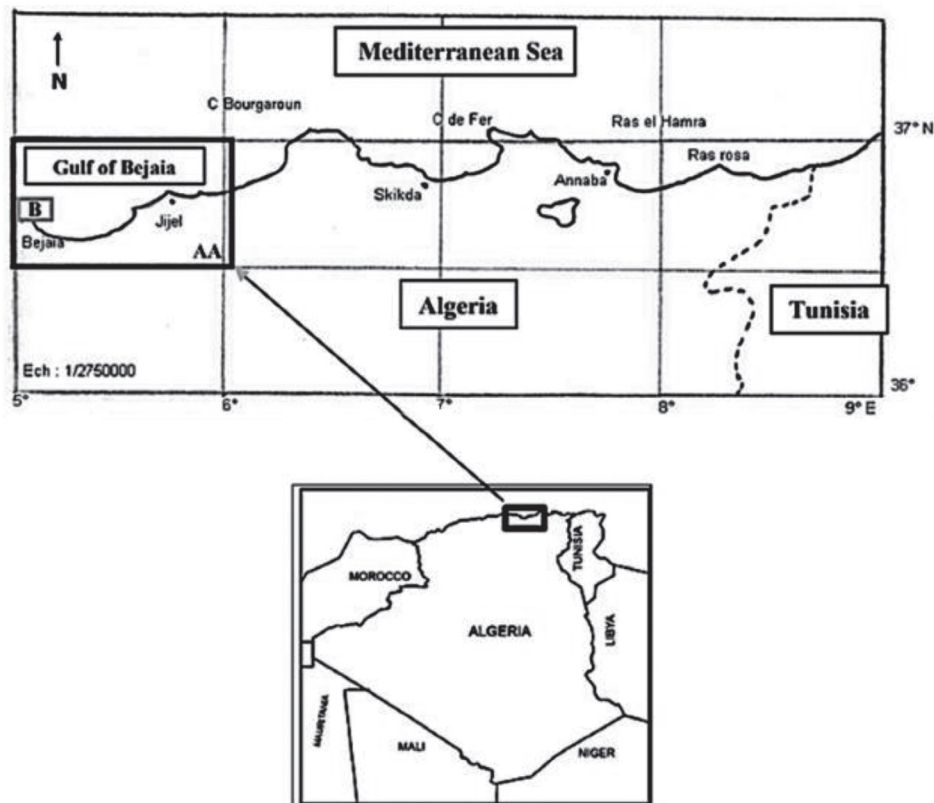
Figure 1. Location of the Gulf of Bejaia (Ramdani et al. 2021).

total of 500 specimens of *M. barbatus*, ranging between 98 to 225 mm in total length (TL) were examined. The total and standard length and weight (W) of all specimens were measured to the nearest 0.1 mm and 0.1 g, respectively. Sex was identified.

Sample examination
Any macroscopic abnormalities were registered: vertebral deformities, fins, gills, skin, mouth, eyes, liver and gonads malformations. Morphological abnormalities were photographed with a digital camera Fujifilm 16 Mega pixel.

Radiographic examination
Two specimens have shown vertebral anomalies. They were then subjected to photography and radiography. A normal specimen caught from the same fishing locality was also radiographed and used for comparison. Radiological images were also taken in both lateral and dorso-ventral positions of each fish to show the type of the malformations by Standard radiograph Stephanix ex 600.

Using the radiographs
To assess the degree of abnormality in the anomalous individuals, we measured the height of the curvature of the spinal column (HC). This is the distance between the tangent to the apical vertebra and a straight line passing through the base of the two vertebrae that limit the curvature. The depth of curvature (DC) was calculated with the following formula: $DC = (HC / SL) \times 100$ (SL = standard length fish) used by Louiz et al. (2007). The angle of vertebral abnormalty was measured from the deformity's centre.

Histological study
The target tissues including the white nodal on the caudal fin, asymmetric testicles, ovarian affected tissue, and cheek congested tissue were prepared for the histological examination by fixing in 10% neutralized formalin solution. The samples were then dehydrated in increasing ethanol concentrations (70 to 100%), cleared in xylene and embedded in paraffin wax. Histological sections of 3 μm thickness were then performed using a Leica RM2025 rotary microtome. Sections were mounted on glass microscope slides, stained with Mayer's haemalun-eosin, and mounted with Canada balsam. They were then examined and photographed by light microscopy with an integrated camera.

RESULTS

Cheek congestion
A 155 mm (TL), 115 mm (SL), and 36.4g (W) fish showed clear signs of erythema and swelling in the left cheek (Figure 2 A). There were no other anomalies in necropsy.

Histopathological findings revealed multiple lesions. A marked inflammatory response within the dermal connective tissues was detected. Fibrosis and melano-macrophage centres (MMCs) infiltrating underlying adipose and muscular tissue were also identified. Necrosis of muscle fibres disturbing the normal tissue was also recognized. Alongside these lesions, no infectious agents were found (Figures 2 B, C, D, E).

Skeletal anomalies
Spinal deformities
The measurements of two abnormal fishes were 165 mm (TL), 132 mm (SL), and 47.1 g (W) for the first one, and 180 mm (TL), 140 mm (SL), and 74.8 g (W) for the second abnormal fish. The normal specimen measured was 164 mm (TL), 131 mm (SL), and 49.6 g (W).

Compared to the normal column radiographs (Figures 3 A, B) radiological imaging indicated the presence of three types of curvatures observed in the two specimens of *M. barbatus* examined in this study: scoliosis (S-shaped vertebral column), lordosis (V-shaped vertebral column), and kyphosis (Λ-shaped vertebral column). Morpho-anatomical examination revealed no deformities in the mouth or fins.

In the first case, scoliosis was visible externally. The fish's spine was twisted sideways (Figure 3 C). The vertebrae (v) involved in this scoliosis were the 9–16th v. The 12th v was located at the centre of the deviation. The

value of the angle "A" was 176° and the depth of the curvature was 2.8 mm. Ribs, intervertebral spaces, and vertebral thickness were all normal (Figure 3 D).

In the second case, externally, the fish appeared to be compressed when compared to the normal fish. Lateral deviations with three humps in different sizes of the vertebral column were observed. Besides every two humps, the fish revealed a concavity. The lateral line was disrupted in several places (Figures 3 E, G). According to X-ray radiographs (Figures 3 F, H), the whole spinal column's vertebrae appeared to have been affected by this defect and showed varying degrees of abnormalities. This teratology consisted of a complex spinal column deformity, a continuous case of kyphosis-lordosis-scoliosis from the head to the tail. The vertebrae forming the 1st kyphotic arch were the 1st–8th v, with the 4–5th v at the top of the arch, followed by the 1st lordotic arch containing the 5–14th v, with the 9th v at the bottom of the arch. The lateral side of the spinal column showed double lateral deviations (F) and (G). The 1st scoliotic arch comprised the 12–17th v, and the 2nd scoliotic arch included the 18–23th v. The 2nd lordotic arch (D) involved 15–20th v, with the 17–18th v located at the bottom of the arch. It was restricted between the 2nd and the 3rd kyphosis arches (C) and (E). The vertebrae forming the 2nd kyphotic arch were the 10–17th v. The 3rd kyphotic arch comprised the 18–23th v, with the 14th v and the 20th v at the top of the arch, respectively.

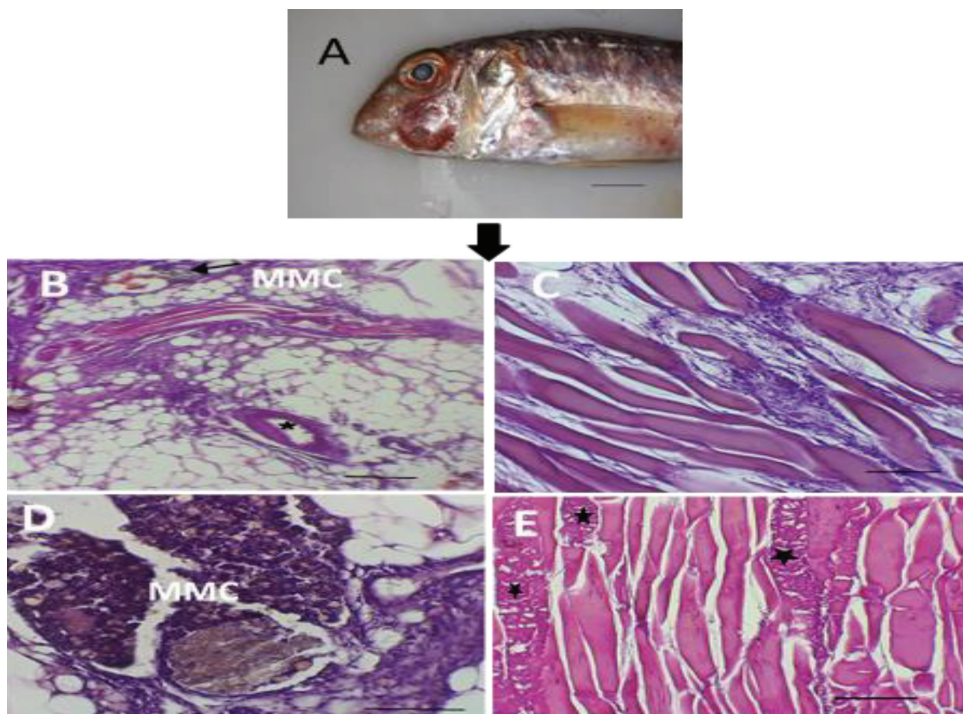The angles "A", "B", "C", "D", "E", "F", "G" in the 2nd specimen had the following values: 98°, 118°, 142°,

Figure 2. Photographs showing congested cheek. **A:** Fish with congested cheek; B: Fibrosis and inflammatory cells infiltrating underlying adipose and muscular tissue. Melano-macrophage centres (MMC). Gross vessel (black asterisks). C: Fibrosis and inflammatory cells infiltrating underlying muscular tissue. D: Melano-macrophage centre (MMC) infiltrating underlying adipose tissue. E: Necrosis of muscle fibres (black asterisks). Scale bar: **A** = 1 cm, B, E = 100 μm, C, D = 50 μm.

144°, 153°, 133°, 144°, respectively, with depths of 11.57, 11.43, 6.79, 5.21, 4.5, 5.79, 4.36 mm, respectively.

Irregular intervertebral spaces and vertebral coalescence in different regions have been detected. This includes the 11–14th, 15–17th and 18–19th vertebrae. Vertebrae fusion has been identified at the 19–21thv. Many deformed neural and haemal spinesin lordotic and kyphotic arches have been observed.

Caudal fin abnormalities
Caudal fin deformity was visible on the fish body immediately. Anomalous caudal fin has been observed in 13.2% of specimens (130–178 mm (TL) and 21–74.5 g (W)). Different caudal fin anomalies (asymmetric fins, wavy rays, reduced fins, deformed shape) have been also observed (Figures 4 B, C, D).

Lymphocystosis
Externally, the infected female fish with measurements of 140 mm (TL), 114 mm (SL), and 29.6 g (W) showed a white nodule with the average length and width of 3

mm and 2 mm, respectively. The nodule was multilobulated, exophytic, presenting a cauliflower-like cutaneous mass protruding from the caudal fin of fish. No significant internal signs were detected after necropsy (Figure 5 A).

The histological slides revealed that the lesions collected from the caudal fin of the infected *M. barbatus* exhibited pathognomonic signs of disease: cytomegaly of dermal fibroblast cells, with a granular cytoplasm, contains basophilic inclusions (Figure 5B), surrounded by a hyaline capsule (Figure 5C). No significant inflammatory process was detected around the cysts.

Gonad abnormalities
Testes asymmetry
One male fish with the measurements of 152 mm (TL), 123 mm (SL), and 39.1 g (W) showed a noticeable disproportion in the size of both lobes. The right one revealed swelling and shortening (Figure 6 A). Both lobes were distinctly separated, and no microscopic disorders in this testis were noticed.
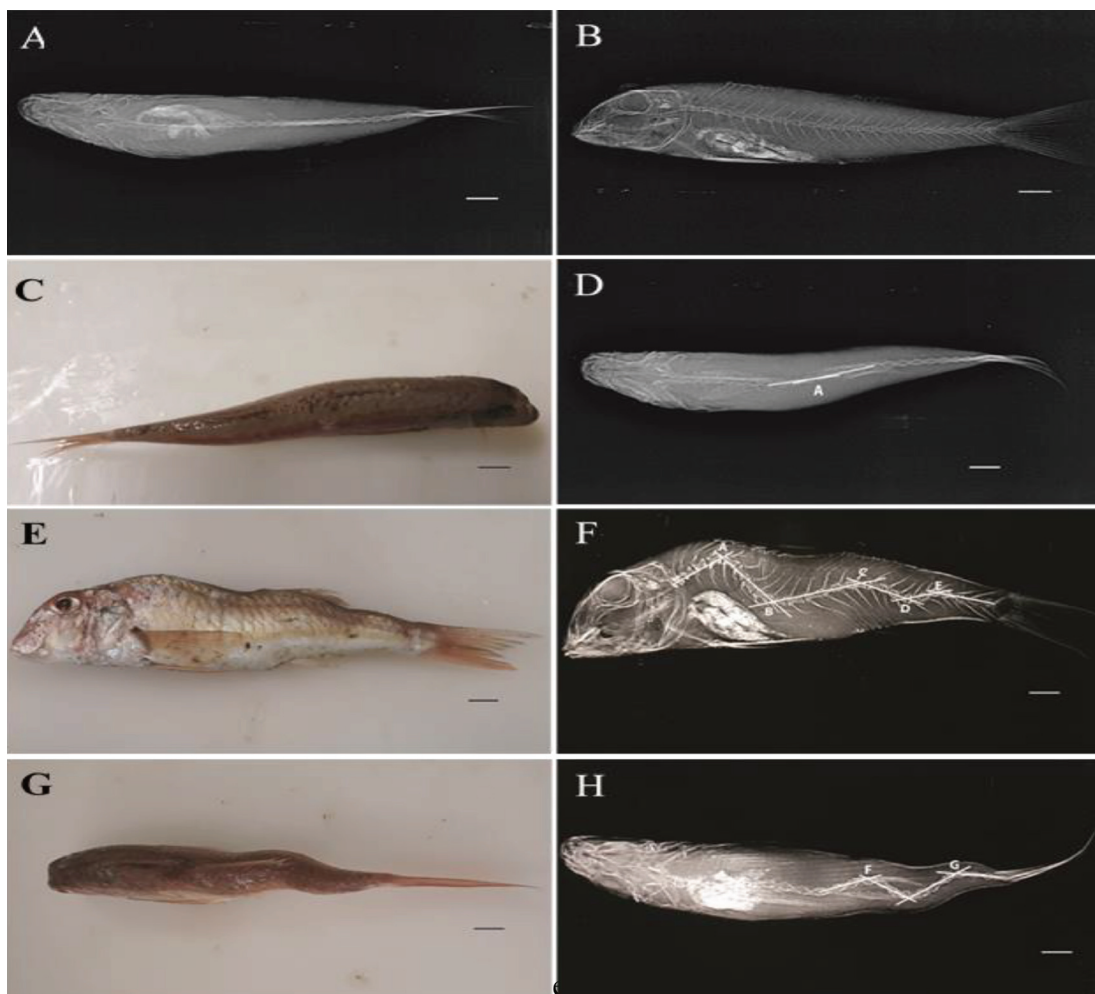

Figure 3. Photographs illustrating spinal deformities in *M. barbatus*. A, B: Radiographs showing normal vertebral column. C, D: Specimen showing scoliosis. E, F, G, H: Specimen showing a complex spinal column deformity (E, F: kyphosis-lordosis; G, H: double scoliosis). Scale bar = 1 cm.
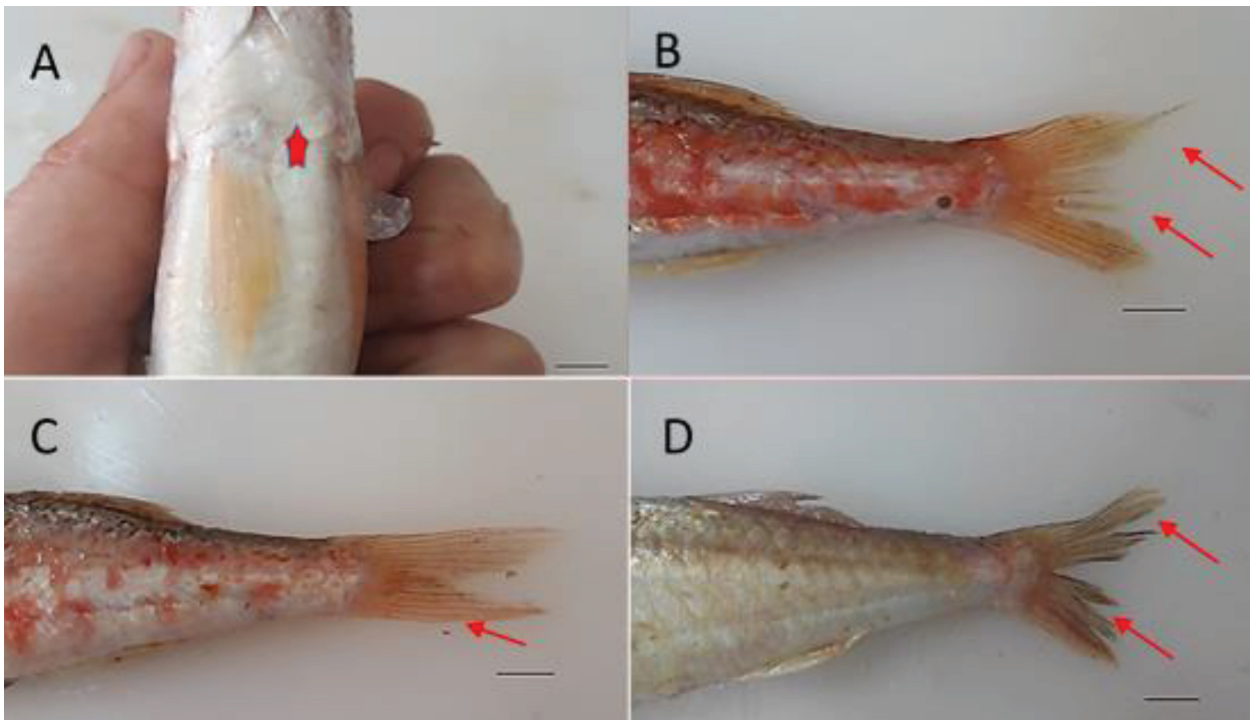
Figure 4. Photos showing fin deformities. A: Missing pelvic fin (red asterisk). B, C, D: Caudal fin deformity (red arrow). Scale bar = 1 cm.
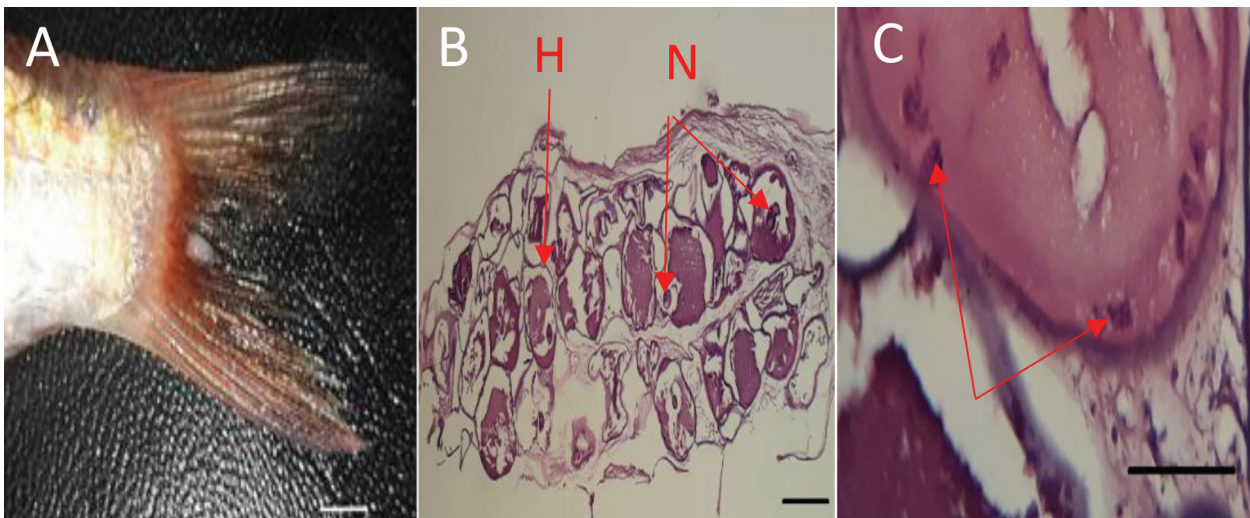

Figure 5. Photos of caudal fin showing lymphocytosis. A: Caudal fin infected by lymphocystis cells. B: Histological view of lymphocystis cells (N: nucleus; H: hyaline capsule). C: Photo showing bodies inclusions in infected cells (see red arrows). Scale bar: A = 0.5 cm; B = 300 μm; C = 50 μm.

Ovary malformation

One female fish in ovulation phase, with the measurements of 175 mm (TL), 147 mm (SL), and 66.7g (W) has shown asymmetric lobes. Its left lobe had an ovary cyst with a mild strangulation from the normal part which was more transparent than the whole organ. Yellowish masses like a group of mature ovocytes were also observed (Figure 6 B).

Histopathological examination indicated a mature ovary with a disorganized architecture. The presence of atresia foci and melano-macrophage centres has been noted at this stage of the study (Figure 6 C). Unknown crescent-shaped structures were also observed (Figure 6 D).

DISCUSSION

Abnormalities are well documented in fish populations from different parts of the world (Sindermann et al. 1978; Divanach et al. 1996; Smith et al. 2002; Gavruseva 2020). Fish anomalies are frequently easy to identify macroscopically and thus offer an important advantage
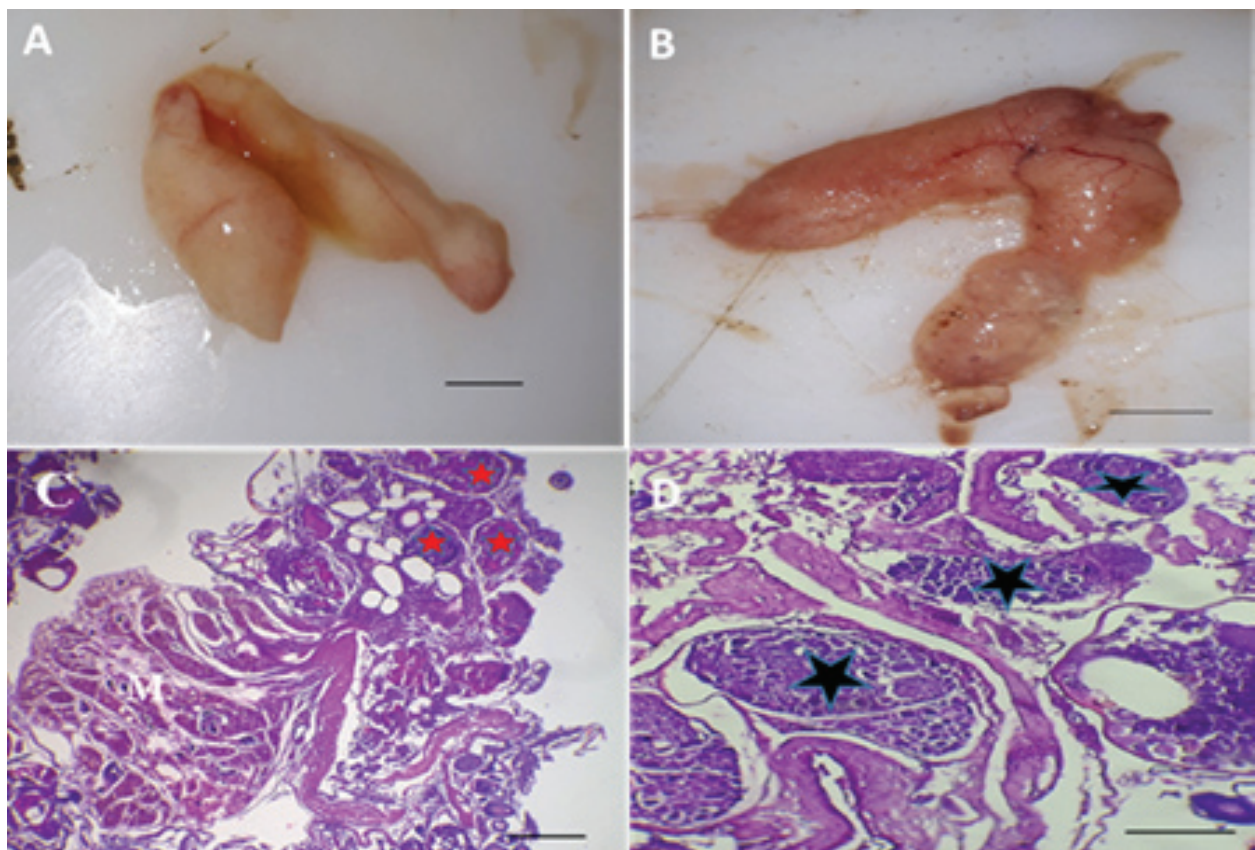
Figure 6. Photos showing anomalous gonads. A: Asymmetric testis. B: Ovary cyst. C: Melano-macrophage centres (M) and ovocyts atretic foci (red asterisks). D: Unknown structures (black asterisks). Scale bar A, B = 1 cm, **C** = 400 μm, D = 100 μm.

over other approaches, especially for researchers operating in the field without access to laboratory equipment (Jawad and Abed 2021). However, there is no published information concerning malformations on *M. barbatus* of the Algerian coast. Here, we report our first observations on deformities in this species in this area.

In adult farmed rainbows, some dermal changes and histopathological similarities to our case (Figure 2) have been reported. Typically, only one side of the head was affected. This is the case of swelling and redness of the cheek/jaw region, extensive inflammation surrounding the cartilage, infiltrated muscular and connective tissues, which are visible microscopically (Cano et al. 2021). The inflammatory process may be initiated by biological, chemical, or physical agents. It may persist and develop into a chronic reaction because an acute inflammatory reaction cannot be resolved, either because of the injury-causing agent's persistence or some interference with the body's normal healing process. When the inflammatory injury involves tissues that cannot regenerate or when there is a significant amount of fibrin exudate, the area of exudate becomes covered in connective tissue, which transforms it into a mass of fibrous tissue. The damaged tissue is gradually reabsorbed and replaced by fibrosis (Kumar et al. 2004).

Adventitious melano-macrophage centres (MMCs) can develop within maturing chronic inflammatory lesions (Agius and Roberts 2003). In teleost fish, MMCs, also known as pigmented cells, include melanin, ceroid/lipofuscin, and haemosiderin as intracytoplasmic pigments (Wolke 1992), which have been described as densely packed clusters of macrophages with a variety of inclusions and are commonly found as distinct groups in haematopoietic and some other soft tissues (Agius and Agbede 1984). These MMCs are commonly used as biomarkers for diseases, toxic pollutants, and environmental destruction (Agius and Roberts 2003).

This chronic inflammation probably prevents gaping and jaw mobility, ultimately preventing feeding. Without additional tests and in the absence of a pathogen identified on the cheek lesions many probable etiologies could be attributed: viral, bacterial, and it may be a trauma. A possible link to water contaminations was also taken into account. Fish exposed to contaminated waters can bioaccumulate heavy metals. At a certain degree of exposure and absorption, all heavy metals are potentially toxic to most organisms (Gümgüm et al. 1994). According to Aissioui et al. (2021), *M. barbatus* had the highest Cd and Hg levels than other benthic fish species found along the Algerian coast, in which Bejaia

coast has shown the highest average concentrations of Hg. This is most probably due to *M. barbatus's* burrowing attitude, which exposes it to contaminants through the sediments.

Naturally occurring skeletal abnormalities are infrequent in wild fish populations, either because they are less abundant or because the abnormal fish are less viable in their natural habitat (Gavaia et al. 2009). Skeletal abnormalities are a complex mixture of different bone disorders and are not well understood (Berillis 2015). Many parts of fish body can be affected by these abnormalities: cranium, vertebral column, ribs, and fins (De La Cruz-Aguero and Perezgomez-Alvarez 2001). Fish anomalies can be divided into two principal categories: severe ones that impact fitness, and less severe ones that don't affect survival (Jawad et al. 2019). Kyphosis, lordosis and scoliosis are the most prevalent forms of deformity seen in fish, which can cooccur in a variety of ways (Arbuatti et al. 2013).

Previously, spinal malformations, fusion, coalescence, hyperostosis and some minor abnormalities were observed in one specimen of red mullet collected from Izmir (Jawad and Akyol 2018). Another one showed a lordosis-kyphosis-ankylosis case collected from the northern Aegean Sea, Turkey (Jawad et al. 2018). There are numerous scoliosis cases similar to our first case that have also been reported for other species with variable degrees of severity (Wakida-Kusunoki et al. 2014; De Azevedo et al. 2016; Jawad et al. 2019). Our second case had similarities to those described in reared *Sparus aurata* (Afonso et al. 2000), *Zosterisessor ophiocephalus* and *Aphanius fasciatus* from the Gulf of Gabes (Messaoudi 2008, 2009). The spinal fusion seen in this specimen is often characterized by the emergence of changes in the extracellular matrix contents (ECM) and mineralization of intervertebral sites and arch centre (Ytteborg et al. 2010 a). The current specimen may have faced unfavourable environmental circumstances that caused this malformation. Kranenbarg et al. (2005) suggested that the observed alterations in the vertebral bone structure might be caused by either (1) distorting the normal vertebral form or (2) actively remodelling the vertebral osteoid bone as a result of external influences.

Since the affected specimens were adults, the case of deformation was not lethal, but it undoubtedly had some impacts on mobility. The swimming ability of normal fish and of fish with skeletal abnormalities were noticeably different (Basaran et al. 2007). The paired fins of fish involving pelvic fins are primarily concerned with the generation of vertical forces, and thus have a significant impact on pitching (rising and diving) equilibrium (Harris 1937). On one hand, there are numerous cases of absence of one pelvic fin or both pelvic fins (Valladolid and Przybylski 2000; Jose et al. 2020; Jawad

and Abed 2021). On the other hand, various abnormalities concerning the caudal fin were described in many fishes: double caudal fin, tri-lobed caudal fin, partial tail, separate extra caudal fin rays, undulation of the caudal fin, reduced or asymmetric fins (Tyler 1970; Jawad et al. 2010; Berillis 2015; Quigley 2015; Jassim et al. 2018). Any abnormality in the caudal fin reduces the flexibility of the tail, lowering the fish's performance, including its ability to find food and avoid predators (Jawad et al. 2010). It is evident from the current study that fish teratology is extremely complicated and cannot be attributed to a single reason. Multiple causes can be suggested: genetics (Ishikawa 1990; Berillis 2017), malnutrition as phosphorus deficiency, vitamin C deficiency, vitamin K deficiency and hypervitaminosis A (Berillis 2015), parasites and bacteria (Treasurer 1992; Balebona et al. 1993; Kent et al. 2004; Yokoyama et al. 2005; Pasnik et al. 2007;). According to Raj et al. (2004) environmental factors are also important and can engender deformities by altering the biological mechanisms needed to keep the biochemical integrity of bone, or by neuromuscular effects, which cause deformities without causing a chemical change in vertebral composition, such as temperature (Ytteborg et al. 2010 b; Amoroso et al. 2016), water velocity (Backiel et al. 1984), light (Agniwanshi 2018), and heavy metals can also play a role (Sfakianakis et al. 2015; Messaoudi et al. 2009). It has also been reported that deformed fins, including the lack of pelvic and caudal fin anomalies, have previously been linked to inherited or post-natal anomalies and environmental stressors (Slooff and Van Krejl 1982; Valladolid and Przybylski 2000; Jawad and Abed 2021). Skeletal abnormalities pose a serious threat to fish survival and growth. The morphological appearance of the commercial fish is impacted by these deformities, which lowers their market value (Berillis 2015).

In the aquatic environment, lymphocystis disease (LCD) is one of the oldest and most ubiquitous fish viruses (Borrego et al. 2017). While most of the LCD research has concentrated on ornamental or aquaculture-relevant species, little attention has been paid to the phenomenon's manifestation in wild populations (Palmer et al. 2012). It is the first detection of LCD case in fish populations in Algeria. There are no epidemiological findings related to the LCD causative virus in this part of the Mediterranean Sea. It is also the first report of lymphocystis disease in red mullet (*Mullus barbatus*) in the world, although its congener (*Mullus surmelutus*) was diagnosed in Plymouth waters in 1949 (Alexandrowicz 1951). In this study, the diagnosis of lymphocytosis infection was made by histopathological study, but it was impossible to identify the species of lymphocystis virus causing the infection, because of lack of the needed molecular identification equipment.

Clinically and histopathologically, the disease affecting *Mullus barbatus* is very similar to other cases of LCD that have been reported in previous different fish species (Alexandrowicz 1951; Palmer et al. 2012; Cherif et al. 2020). The giant cells can be found isolated or in clusters and frequently appear as nodules beneath the epidermis, particularly on the fins (Russel 1974). The presence of only one single small node of LCD in this case is probably due to the fact that the fish in the present study are young and the disease had a limited life cycle (Sheng et al. 2007). The lack of any inflammatory response in other fish species has been previously documented (Durham and Anderson 1981; Perretta et al. 2020) and this can be due to the fact that the disease is still developing (Russell 1974; Perretta et al. 2020).

It has been reported that fish under increasing chemical contaminants or other anthropogenic stressors are more vulnerable to lymphocystis (Overstreet 1988; Vethaak 1996; Hogan et al. 2018). According to Vethaak (1996) pollution exposure could increase lymphocystis prevalence, probably due to fish immunosuppression. LCD infection has been previously noticed to be responsive to temperature (Hossain et al. 2009). The seasonal prevalence of the disease has also been reported (Kitamura et al. 2007). Nigrelli (1952) and Wolf (1962) have reported that there was a relationship between lymphocystis disease and certain parasites, although LCD infections are generally benign and self-limiting. The affected fish could become unmarketable due to the warty appearance (Borrego et al. 2017).

Due to its vulnerability to external perturbations reproduction is a sensitive environmental indicator (Rajasilta et al. 2016). Many types of gonads malformations such as hermaphroditism, atrophy, compartmentations, and asymmetry have been described for a number of fish populations in both sexes (Bernet et al. 2004; Hliwa et al. 2011; Sopinka et al. 2012; Rajasilta et al. 2016). Rajasilta et al. (2016) have reported that asymmetry was the most frequent gonad type that deviated from normal in the Archipelago Sea, which was observed during the entire study period at varying but low frequencies.

Abnormalities concerning the development of the reproduction system may occur as early as in the stage of gonad formation (Hliwa et al. 2011). Since asymmetries were prevalent in the coregonids of Lake Thun and two nearby lakes, it is believed that they represent a natural component of the variation in whitefish gonadal morphology (Bernet et al. 2009). Sopinka et al. (2012) have reported that *Porichthys notatus* males in high-exposure areas exhibited more asymmetry in their testicles, shorter-headed sperm, and fewer living eggs in their nests. However, Rajasilta et al. (2016) have suggested that asymmetric gonads would only be minor deviations from the usual and wouldn't impact the fish's ability to reproduce regularly. The causes of such anomaly are still unknown, but it has been shown that pathogens, parasites, and chemicals that produce crypto-endocrine or toxic effects can all have a significant impact on how fish gonads form and behave (Hliwa et al. 2011; Sopinka et al. 2012; Rajasilta et al. 2016).

In addition to what has already been reported (pathologies, toxic pollutants, and environmental degradation), MMC may also develop during ovarian atresia (Ravaglia and Maggesse 1995; Kumar and Joy 2015). Although it is more common in regressing ovaries during the post spawning period, follicular atresia is a normal part of fish oogenesis and is seen throughout the ovarian cycle (Ganias et al. 2008). Lower annual fecundity and even infertility in fish, both wild and farmed, can result from an increase in atretic rate above physiological levels (Corriero et al. 2021). Follicular atresia has been shown to rise above average rates due to a number of factors such as starvation and different forms of stress (Brraich and Jangu 2015; Corriero et al. 2021). Water pollution can also result in follicular atresia by endocrine-disrupting chemicals (Brraich and Jangu 2015; Chukwuka et al. 2019). Recent studies suggest that also exposure to microplastics can lead to atretic folicles (Yan et al. 2020). Due to the abnormal macroscopic ovary, we suggest that atresia and MMCs were pathological probably caused by aquatic pollution.

CONCLUSION

Fishery management is critical for Algeria, as it is for other Mediterranean Sea countries, on both a social and economic level. Deformities that affect fish cause losses, or customers found it to be unfavourable. However, in order to avoid public health issues, our first priority should be to highlight the main causes of these malformations by more advanced methods and make additional efforts to improve fisheries management.

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