Review of the principal diseases affecting cultured meagre (*Argyrosomus regius*)

Florbela Soares[a*], Ana Roque[b], Paulo J. Gavaia [c,d]

[a]IPMA, Portuguese Institute of the Sea and the Atmosphere, EPPO- Aquaculture Research Station, Av. 5 de Outubro s/n, 8700-305 Olhão, Portugal.

[b]IRTA, C/ al Poblenou SN. 43540 Sant Carles de la Rapita, Spain

[c]CCMAR – Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

[d]Department of Biomedical Sciences and Medicine, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

*Corresponding author: Florbela Soares. IPMA, Aquaculture Research Station, Av. 5 de Outubro s/n, 8700-305 Olhão, Portugal.

Tel:+351 289 715 346

E-mail address: fsoares@ipma.pt

Florbela Soares: orcid.org/0000-0001-6075-2472

Paulo Gavaia: orcid.org/0000-0002-9582-1957

Ana Roque: orcid.org/0000-0001-8717-9186

Abstract

Argyrosomus regius was recently introduced in European aquaculture as a promising species for diversification and with high potential for expansion in the coming years. The reports on pathologies affecting this species are still scarce in the bibliography, however they can severely affect production and result in major economic losses. Some few reports were made on outbreaks and mortalities associated with the presence of bacteria such as *Vibrio anguillarum* and *Photobacterium damselae* subsp. *piscicida.* Although no viral diseases have been recognized as affecting meagre, it is known that meagre can be an asymptomatic carrier of two genotypes of nodavirus, the striped jack nervous necrosis virus and red-spotted grouper nervous necrosis virus. Up-to-date, parasites affecting meagre are included in the genera Monogenea, Nematoda and Dinoflagelate, but without major mortality outbreaks associated to this type of infections. Osteological deformities have been observed in all life stages particularly affecting the axial system in larval and early juvenile specimens, with a higher incidence in the vertebral column, being affected by vertebral fusions, lordosis and kyphosis. With this review the authors provide an overview of all the pathological and non-pathological diseases affecting aquaculture produced meagre and provide a comprehensive overview of possible problems for the industry.

Key Words: Aquaculture, bacteria, bone deformities, parasites, pathology

Running title: Revision of pathologies affecting meagre

Introduction

In the last decade, there was an increase in the importance of meagre (*Argyrosomus regius,* Asso 1801) to Mediterranean aquaculture (Kružić et al. 2016) with the production in Europe reaching 14,000 tons in 2014 (FAO). Several biological features make meagre an interesting species for aquaculture, including its fast growth in captivity, good feed conversion ratios, the capacity to withstand diverse environmental conditions, controlled spawning in captivity and no maturation during ongrowth (Duncan et al. 2013). Nevertheless, the information regarding the pathologies affecting the species and the optimal conditions for its production are disperse. This fact justifies the need for a review about the pathologies that can affect this species similarly to what happened to other marine species with the intensification of their production. Therefore, it is required an integrated approach to collect data and provide fish farmers with a tool for managing and monitoring fish under different stages of production in order to ensure the quality of the produced fish, allowing for higher survival rates by preventing the incidence of diseases and skeletal deformities.

It has been stated that the major diseases affecting farmed fishes vary in prevalence according to the geographic latitude of countries around the Mediterranean basin, while the methods and the culture conditions used greatly influence the types and severity of diseases (Colorni, 2004). “Old” pathogens (Lymphocystis and encephalitis viruses, “*Pasteurella*” *piscicida*, *Vibrio* spp., *Amyloodinium ocellatum*) still cause serious economic losses in fish production, and diseases once considered sporadic are now emerging, like the ones caused by *Mycobacterium marinum*, *Streptococcus iniae*, *Lactococcus garvieae*, *Enteromyxum* [formerly *Myxidium*] *leei* and *Kudoa* sp.

Aquaculture produced meagre seems to be less susceptible to diseases than other marine species produced in aquaculture, despite the fact that the number of bibliographic records are more abundant for species already long established in the industry. In addition, this species presents a low incidence of larval deformities, when compared with the levels commonly reported in other Mediterranean fish larvae (Le Vay et al. 2007) such as gilthead sea bream (*Sparus aurata*

Linnaeus*, 1758*) (Georgakopoulou et al. 2010), European sea bass (*Dicentrarchus labrax* Linnaeus, 1758) (Koumoundouros et al. 2002) or Senegalese sole (*Solea senegalensis* Kaup, 1858) (Gavaia et al. 2002). Moreover, its response to stress indicate that it follows the general patterns of stress response as for most fish species, though there seems to be a clear species-specificity in cortisol, glucose and lactate stress mediated response in the magnitude, timing and duration. Among Mediterranean species, a very high response was observed in European sea bass while dusky grouper and meagre showed a very low response. This classification was based on a comparison of seven Mediterranean fish species, where 30 fish *Sparus aurata*, *Dentex dentex*, *Pagellus erythrinus*, *Diplodusm puntazzo*, *Epinephelus marginatus*, *Argyrosomus regius* were chased (5min) and then exposed to air (1-1.5 min), after which they were blood sampled. A control of five fish per species was anesthetized and sampled without previous chasing event. Plasma was separated from blood cells and analysis of cortisol, glucose and lactate were performed. Sea bass, in all parameters, had a higher and more intense reaction when compared with the other species. In the case of cortisol, seabass presented levels 20 times higher in the plasma than meagre. Fanouraki et al. (2011) attributes this variability to differences in the metabolic needs and specific habitats of the fishes, unrelated to genetic differences.

Regarding infectious diseases, the presence of several parasites has been reported and some bacteria have been associated with outbreaks of disease in meagre. Up to date, and to the best of our knowledge, there are hardly any reports describing the existence of viral diseases of meagre and despite some reports on the presence of nodavirus, no disease outbreak in meagre has been associated to its presence, and meagre is believed to be simply a carrier.

Bacterial diseases

Vibriosis

Chronic vibriosis has been reported in meagre and thought to be caused by *V. anguillarum* (Garcia et al. 2013) causing 1.5 % mortalities in cages close (2 miles) to where acute vibriosis in seabass are developing. Clinical signs displayed by the fish included reddened fins and areas around vent and mouth and also loss of appetite. Flumequine, oxytetracyclines, sulfonamides (+trimethoprim) and florfenicol are good options for vibriosis treatment (Garcia et al. 2013).

Larval vibriosis was detected in the Algarve (Portugal) in hatchery reared meagre, which presented no pathologies until 30 days after hatching. After that age, a bacterial disease caused by Vibrionaceae was identified, resulting in larval mortalities reaching 33.7% (Soares et al. 2012a). In the same facility, broodstock were also affected by a disease caused by Vibrionaceae when temperatures of the water were from 14 to 24ºC, it is not reported whether the it refers to one or more outbreaks (Soares et al. 2012a).

In Spain, *V. anguillarum* serotype O1 has caused disease in meagre raised in offshore cages since 2005, with an approximate monthly mortality of 1% in fish around 1 kg, and 30% in fish around 150g (Haenen et al. 2013).

Vibriosis has also been reported in meagre cultured in Egypt (West Alexandria), where cage-cultured fish, with a body weight range from 50 g to 3 Kg were sampled, including 100 specimens showing clinical signs. Those fish presented erythema and reddening of the mouth with opaqueness on the eyes, hemorrhagic caudal and anal fins, erosions at caudal fin and reddening around the anal opening, while others showed ulcerations at the caudal peduncle with hemorrhagic caudal fin. Internally, meagre showed hemorrhagic ascetic fluid and congestion of gills while other fish showed whitish nodules in the liver. From the bacterial analysis, the species most abundantly found included *Vibrio alginolyticus* (10 % of the 50 isolates identified from meagre), *Tenacibaculum maritimum* (8 %) followed by *Vibrio harveyi* (6 %) (Saad, 2013). However, the study by Saad (2013) did not include any challenge work for which the isolates could be positively linked to the clinical signs, though they are consistent with what is observed for vibriosis in fish.

The presence of *Vibrio tapetis* has been reported by Cardenas (2011) in meagre from ongrowing experiments at IFAPA (Spain). However, and since it is a bivalve pathogen, an experimental infection made with this agent to confirm the potential of *V. tapetis* to affect meagre proved unsuccessful.

Photobacteriosis or fish pasteurellosis is known to be caused by *Photobacterium damselae* subsp. *piscicida* (Ph.d.p.). It was reported for the first time by Bottari et al. (2009) that meagre is sensitive to Ph.d.p, although this was only observed during a challenge test. That experimental challenge was performed by intramuscular injection or intraperitoneal injection of juvenile fish (average weight 80g) with a bacterial density of 10^5 per fish and mortalities were recorded from the 2nd to the 6th day. Fish injected intramuscularly and intraperitoneally displayed 70% and 80% mortality respectively. Control fish had no mortality. This challenge was performed at the same time in European sea bass as a positive control, and in common dentex (*Dentex dentex* Linnaeus, 1758). Meagre was shown to be the most sensitive species to Ph.d.p., displaying the highest mortalities in the three species used for that study (Bottari et al. 2009) indicating that exposure to this pathogen in aquaculture conditions or in the wild may be responsible for causing high mortalities. The injected bacteria were re-isolated from the internal organs of all the dead fish and the histological exam showed focal necrosis of the lymphoid organs, especially in the spleen and head kidney. Strategies for immunization against Ph.d.p. are available, either by immersion vaccination with bacterins, that proved highly efficient, or by injection of purified membrane or extracellular proteins produced by Ph.d.p., and were proven to provide protection in other Mediterranean species like Seabass (Barnes et al. 2005). These vaccination strategies should be tested in meagre in order to understand if they would be effective in preventing possible high mortalities associated to disease outbreaks. Currently, the development of vaccines for meagre is not happening since production levels do not justify the investment, however not only it is a new promising species as its production increases yearly

(FAO, 2017 - http://www.fao.org/figis/servlet/TabSelector#lastnodeclicked- accessed 21/11/2017) indicating that sooner or later vaccines for meagre must be considered.

Mycobacteriosis

There is scarce information regarding marine mycobacteriosis, with only one case reported in Turkey, affecting adult meagres farmed in cages in the Aegean Sea. Diseased specimens collected were showing lethargy, weakening, deformed body, lack of appetite, exophthalmia, abdominal swelling and dermal lesions. Internal organs showed the presence of nodular lesions in the liver, kidney and spleen. Homogenates from the internal organs revealed the presence of *Mycobacterium marinum* and histological analysis of the kidney showed the presence of granulomas necrosis in the centers, surrounded by inflammatory cells. The source of infection could not be identified, though wild fish surrounding the cages were negative. A hypothesis was raised that either food was contaminated, or the fish were infected before being stocked in the cage (Avsever et al. 2014). Nevertheless, screenings for the presence of *M. marinum* should be performed as a precaution to prevent the introduction of infected specimens in aquaculture operations, which can cause significant losses in productivity. Again, no experimental challenges were performed in order to confirm causality.

Nocardiosis

Meagre has also been reported to be affected by the presence of Nocardia like organisms causing granulomatosis. This pathology was first reported in meagre by Ghittino et al. (2004) in Italy. A grow out farm in Greece was affected by a nocardiosis outbreak where the disease was present throughout the year, affecting mainly 1 to 2 year old fish, with a reported mortality of 1 - 4%. Fish presented multiple skin lesions with ulcerations and necrosis and the surface of the internal organs displayed yellowish-white nodules with 0.1–0.5 cm in diameter that where observed to manifest progressively as calcified and necrotic organs. Histopathology revealed a systemic granulomatous inflammation and specific staining revealed the presence of Gram-positive Nocardia-like organisms. Those bacteria were grown in blood agar and a preliminary identification was made using APIzym. Nocardia identity was later confirmed by PCR of the 16S rRNA (Elkesh et al. 2013). There are, to our knowledge, no further reports on this outbreak or others caused by Nocardia-like organisms.

In Spain systemic granulomatosis affects almost 100% of meagre cultured populations, but is not associated with high mortalities (Katharios et al. 2011a). In all the reported cases the development of the disease has been associated with a nutritional problem. Further research is needed to fully understand the implications of this widespread disease and the environmental or culture conditions that potentiate its manifestation.

Viral diseases

Nodavirus

Although nodavirus is not frequently reported in meagre, it has been detected in wild specimens inducing no apparent symptomatology, which raises serious concerns. Since meagre is often cultured in the same facilities as European seabass, a species that is severely affected by this virus, and may cause significant losses to producers. Therefore, a decision was taken to review the disease and the specific reports in meagre.

Infection with Nodavirus induces a disease initially named viral nervous necrosis (VNN), however currently the OIE (Office International des Epizooties) recommends the use of viral encephalopathy and retinopathy (VER) (OIE, 2003). Generally, the clinical signs of VER include uncoordinated or erratic swimming behavior, with affected fish unable to keep their balance, abnormal behavior and difficulties controlling swimbladder which tends to be hiperinflated. Additionally, ocular and skin pigmentation were affected (Munday et al. 2002). Histologically, VER induces lesions the central nervous system, with vacuolization and necrosis of brain tissue. The retina is one of the most affected tissues, presenting lesions in the nuclear layers with vacuolization. All these lesions are responsible for varying degrees of neurological disorders in the affected fish (Hodneland et al. 2011).

The disease affects commercial size fish, affecting high numbers of individuals and inducing significant mortalities (Aspehaug et al. 1999; Le Breton et al. 1997; Nylund et al. 2008), but it also affects early stages like fry and fingerlings, leading to rapid mortality (Munday et al. 2002).

Nishizawa et al. (1997) classified the Betanodavirus genus in four genotypes: striped jack nervous necrosis virus (SJNNV), red-spotted grouper nervous necrosis virus (RGNNV), tiger puffer nervous necrosis virus (TPNNV) and barfin flounder nervous necrosis virus (BFNNV). It is generally accepted that viruses belonging to different genotypes show a different range of hosts. Thus, RGNNV infects a wide variety of warm-water fish species; BFNNV is only detected in cold-water marine fish species, while TPNNV only infects one fish species. SJNNV is now known to affect species frequently cultured in South European aquaculture, such as Senegalese sole, gilthead sea bream and European sea bass (Cutrin et al. 2007; Olveira et al. 2008, 2009), though for many years it was only recognized in a few Japanese fish species (Munday et al. 2002; Nishizawa et al. 1997)

Current reports of viral nervous necrosis virus in Southern Europe, either in the Atlantic coast or in the Mediterranean basin, refer to the presence of the SJNNV and RGNNV genotypes (Bovo et al. 1999; Ciulli et al. 2007; Cutrin et al. 2007; Olveira et al. 2009; Skliris et al. 2001).

Wild meagre has been shown to be an asymptomatic carrier of viral nervous necrosis virus (with a prevalence of 46.87 to 56.25%) where two genotypes could be detected in coexistence, the SJNNV and RGNNV (Lopez-Jimena et al. 2010).

Parasitic diseases

Limited data exist on parasitic diseases of meagre, with most records related to those reared in cages in the Mediterranean area which include infections with *Sciaenocotyle* spp. (Monogenea)

(Merella et al. 2009; Ternengo et al. 2010), *Microcotyle pancerii* (Monogenea) (Quilichini et al. 2009), *Benedenia sciaenae* (Monogenea) (Toksen et al. 2007) and *Calceostoma* spp. (Monogenea) (Duncan et al. 2008) (Table 1). Parasites affecting reared meagre in semi-intensive production in earth ponds were described by Soares et al. (2012b) in Southern Portugal. *Amyloodinium ocellatum* and different monogenetic genera (*Lamelodiscus* sp. and *Diplectanum* sp.) were identified in meagre reared under polyculture conditions together with gilthead sea bream, but mortalities were only observed associated with *A. ocellatum* (1.2 % of mortality).

In the western Mediterranean Sea (Sardinia), from September 2007 to January 2008 a massive infection with *Sciaenacotyle panceri* on the gills of cage-reared *A. regius* was associated with emaciation, anemia and mortality (Merella et al. 2009).

Recently, a case of *Diplectanum sciaenae* infecting meagre was reported (Andree et al. 2015). Although three species of Diplectanum have been associated with wild meagre (Oliver 1980), this is the first report in cultured fish. A dead broodstock male showed gill heavily infested with a monogenean later identified as *D. sciaenae*. The fish showed important gill pathologies which authors elaborate that could also provide an easy route for bacterial infections. In nature *Diplectanum* spp. are not very pathogenic but under stressfull conditions they can cause significant damage. The fish in question had been subjected to considerable handling which no doubt caused stress.

Moreover, in caged fish, factors such as high stocking density, low genetic diversity, adverse environmental conditions, and/or poor animal husbandry practices, may increase the susceptibility of hosts, promoting parasite transmission and favoring infections and epizootics by parasites that normally do not affect fish in production (Kent, 2000, Leong and Colorni, 2002) raising the need to have a strict control of environmental parameters, implement high standard aquaculture and zootechnical practices and promote the use of low inbreeding fry.

Noninfectious diseases

Granulomatosis

Systemic granulomatosis was first described in gilthead sea bream culture. In meagre, it occurs during grow out, and it has been associated with vitamin deficiencies in the diet, mainly vitamins from the C and B complexes (Ghittino et al. 2004).

It is caused by the depositing of tyrosine crystals in tissues, predominantly kidney and spleen. A case of this pathology was diagnosed in intensively reared meagre in Italy, and lesions encountered were comparable to those observed in gilthead sea bream (Ghittino et al. 2004). At necropsy, a mild exophthalmos associated with keratitis and cataract, together with reddening of the skin and ulceration. Kidney, spleen, liver and heart were scattered with whitish nodules of different dimensions. Histological examination pointed to a less chronic evolution of the granulomatous process in meagre compared with the one found in gilthead sea bream. Granulomas, which were not surrounded by a connective capsule, were mostly localized in the perivascular areas of the various organs and were associated with a peripheral inflammatory reaction and hemorrhaging. That case was further studied, and upon clinical examination of the specimens most severely affected, weighing 2-3 kg, they showed bilateral exophthalmia and keratitis associated with ulcerative skin lesions, scattered mainly on the head, body sides and abdominal regions and close to the insertion of the dorsal fin. The necropsy showed the existence of granulomatous lesions in the kidney, spleen, liver and heart. The histological exam of the most extensive skin lesions, which were found mainly on adult individuals, showed the presence of necrotic ulcerated areas, often extended into the dermis and infiltrating the muscle layers. In all those sites, multifocal granulomatous lesions were found. Samples were also taken for fungus culture and showed growth from every organ examined with colonies of cottony texture and powdery surface, with the frequent presence of radial grooves, initially white and then evolving shades of green or blue-green, while the edge of these colonies was always white.

Microscopic examination of the colonies showed hyaline and septate hyphae of about 5 µm in diameter. The observations made on those colonies, led to ascribe them to the genus *Penicillium* (Andreoni et al. 2003), and later identified as belonging to the species *P. digitatum (*Manuali et al. 2005). Currently in Greece, granulomatosis is thought to affect 100% of cultured meagre populations. Katharios et al. (2011a) reported that this disease is characterized by multiple systemic visceral granulomas that manifest progressively as calcifications and necrosis in the organs. The etiology is unknown and it is not associated with high mortalities, though affecting the final product and rendering it not acceptable for consumers.

Chronic erosive dermopathy

A pathology similar to chronic erosive dermatopathy was already detected in reared meagre, and suggested to be associated to borehole water usage (Rigos and Katharios, 2010). Although few descriptions of this condition are present in the literature and it is described to affect 100% of the cultured populations, it has not been associated with high mortalities. Chronic erosive dermatopathy is known to cause ulcerations in the skin covering the lateral line canals, resulting in severe disfigurement that makes the fish unacceptable for consumers (Katharios et al. 2011b). The disease is directly associated with the use of borehole water, although the etiological factor is still unknown. The observed lesions disappeared when the fish were transferred to tanks with surface water supply (Baily et al. 2005; Katharios et al. 2011b), however, complete recovery is observed only in the cases where fish were transferred early during development. Schultz et al. (2011) described that the pre-treatment of borehole water was effective in preventing the development of this dermopathy. In this sense, it is recommended that the source of the water used for rearing juvenile meagre is carefully treated and that borehole water is avoided if other supply options are available.

Neoplasia

Reared juvenile meagre developed bilateral or lateral sarcoma inside the opercular cavity (Soares et al. 2011, 2012c). The sarcoma revealed to be reddish and dense, growing from the branchial arch and exerting pressure on the operculum, forcing it to open. Histologically, the sarcoma showed a strong propagation of mesenchymal connective tissue, largely formed by fusiform cells, growing in a solid form with numerous mononuclear cells. Areas of necrosis were also observed. An inflammatory response was associated with that pathology and *Vibrio* species were isolated from the tumor. However, no bacteria were isolated from the liver, spleen or the anterior kidney. After electron microscopy observation, it was discarded a viral origin. Regarding cytogenetic alterations an increase in the aneuploidy level was observed in sarcoma cell metaphase stages when compared with other fish tissues. As far as we know, horizontal transmission of neoplasia does not occur, except in cases were infectious viruses or parasites are the causative factors (Soares et al. 2014).

Skeletal problems

The meagre is a species with rapid growth during larval and post larval stages and shows a fast skeletal development, with a nearly complete number of structures present when larvae reach 12 mm, revealing almost all structures of the axial skeleton calcified and squamation forming (Cardeira et al. 2012). Due to this fast developmental pace, the individuals rapidly reach a length of 4-5 cm (*circa* 50 DAH) and display a fully developed skeletal system and attain the full complement of meristic characters that characterize the species (Table 2).

The vertebral column of the aquaculture produced individuals (Fig. 1 and 2A, 2C) was composed of 25 vertebrae distributed into 5 cephalic vertebrae (vertebral elements that articulate with the skull and display neural arches but no parapophysis), 5 pre-haemal vertebrae (displaying neural arches and parapophysis), 12 haemal vertebrae (displaying neural and haemal arches) and 3 caudal vertebrae (with modified haemal and/or neural arches and spines that help support the caudal fin) including the urostyle (an upward oriented modified vertebral body that articulates with the hypurals supporting the caudal fin rays). In addition, the cephalic and pre-haemal vertebrae articulate with 9 pairs of pleural and epipleural ribs.

Juveniles larger than 4 cm already have enough bone density to allow radiographic analysis of their skeleton using a low energy X-Ray device. This process allows a rapid evaluation of the general status of the skeleton in a group of fish simultaneously and does not require sacrifice of the specimens, only involves a short period of sedation and manipulation, being immediately recovered in clean water and returned to the rearing tanks. Our experience in analyzing meagre juveniles showed that light sedation induced for 1-2 min is well tolerated by the fish and will result in a state of movement cessation for the period of 30-45 sec. needed for soft radiography. The fish can be placed in a polystyrene tray with anesthetic solution for radiography, to avoid air exposure and anoxia, and in this way causing less stress and virtually no mortality after recovery in clean sea water (Castanho et al. 2017).

The bibliographical records for skeletal problems in this species are very scarce. A report of a specimen with 80 cm captured in commercial fisheries, displaying a lateral curvature of the caudal peduncle with a large prominent nodule and showing muscular atrophy is the only reference for deformities in wild captured meagre (Mendes and Moreno, 2001). This deformity revealed to be caused by a fracture that had already formed a healing callus and anquilosis, fusing the affected vertebrae and inducing the shape alteration. The occurrence of lordo-kyphosis together with vertebral fusions was observed to be the most common types of deformities that affect the skeletal system in aquaculture produced meagre, negatively affecting growth, survival, susceptibility to diseases and rendering the specimens no fit for commercialization and negatively affecting the consumer's perception of aquaculture produced meagre. In our experimental large scale production of meagre in earth ponds we have found very few specimens at the end of the production cycle showing severe deformities that affect the external appearance of the fish and can make them improper for commercialization (Soares et al. 2014). However, in the analyses of the skeleton of growing larvae and juveniles it was possible to detect some abnormalities affecting mostly axial skeleton with appearance of fused and deformed vertebrae, alterations on the arches and spines and some minor defects on the appendicular skeleton. At the end of production cycle some specimens have been found showing alterations of the body shape that, after analysis, revealed to be severely affected by vertebral abnormalities and curvatures. In the specimen shown in figure 2B the vertebral column presents a lordosis with an angle close to 90º, displaying fusion of haemal vertebrae 12-13-14 and causing a malformation of both haemal and neural arches. Such condition severely affects the movement and may compromise the neuronal function and the blood flow by compression of the neural chord and of the ventral aorta.

Incidence of deformities in larval stages is mainly located in the axial skeleton with fusions of vertebral centra and arches located in the anterior portion of the vertebral column. The incidence of deformities decreases in the larval population along the production cycle probably due to selection of the deformed specimens (Martins et al. 2013).

Juvenile specimens were analyzed by radiography revealing a high incidence of fish with mildly deformed structures (70%). However, these fish did not present any vertebral curvatures and only showed the presence of fused vertebrae and malformed arches and spines (Fig. 2D), mostly located in pre-haemal and anterior haemal vertebrae. These malformations had no significant effect on the fish external appearance and should be considered as mild, not compromising the normal rearing and future commercialization of these specimens. Nevertheless, in some cases these deformed structures might suffer an aggravation of the deformation and evolve into more severe conditions throughout the production cycle. Conversely, a containment event might occur when fused vertebrae reshape into a vertebral centrum with features similar to normal vertebra but displaying two pairs of neural and haemal arches (Arrows in fig 2D). This process seems similar to the observed in salmon that are affected by where the fused vertebrae present in growing juveniles can undergo a compensatory mechanism that involves the reshaping of the fused elements by bone remodeling and formation of a centrum with similar features to the normal elements (Witten et al. 2006).

Environmental

According to Kir et al. (2015a, 2015b) meagre showed higher sensitivity to acute toxicity caused by nitrite (NO_2-) and ammonia (NH_3) than other marine fishes produced in the Mediterranean. In addition, with higher temperatures the tolerance to these compounds were further reduced, with mortalities observed after 96h of exposure to levels of 49.61 mg/L nitrite at 26ºC, while no mortality was observed at lower temperatures. Similarly, mortalities were increased with exposure to ammonia with a linear relation to exposure time and increasing temperature. It was suggested that safe levels of NO_2^- and NH_3 for juvenile meagre should be adjusted according to the water temperature: 17.7 mg/L NO_2^- and 0.10 mg/L NH_3 at 18ºC, 13.9 mg/L NO_2^- and 0.07 mg/L NH_3 at 22ºC and 4.9 mg/L NO_2^- and 0.04 mg/L NH_3 at 26ºC (Kir et al. 2015a, 2015b).

These data suggest a higher sensitivity of meagre exposed to nitrogenous compounds that are normally present in aquaculture production as metabolic waste products, when compared with European sea bass and gilthead sea bream. In this way, it is suggested that a higher control of environmental and water quality parameters is required to ensure safe levels of nitrogenous compounds in meagre rearing tanks in order to prevent possible stress and mortalities, particularly if the rearing temperatures are above 18ºC.

Conclusion or Future perspectives

With the growing interest in meagre production for the Southern Mediterranean countries, this compilation of the available information on the pathologies that affect this species is a valuable tool to help producers in the prevention of disease occurrence and to minimize economic losses in the future. The reported diseases for meagre included the most common pathologies for marine fish in this production area. The pathology with a wider incidence is granulomatosis, for that reason it is important to investigate its true origin, for adequate prevention. Although the authors considered that cultured meagre seems to be less susceptible to diseases than other marine species (several sea bream species, European sea bass and Senegalese sole).

Acknowledgements

This work was undertaken in the framework of AQUACOR (31-03-05-FEP-03) project, supported by PROMAR Program (Portugal) with FEDER funds and DIVERSIAQUA (MAR2020 16-02-01-FMP-0066) project. This study has been supported in part under the framework of the European Union Seventh Framework Programme project DIVERSIFY (KBBE-2013-07 single stage, GA 603121) titled "Exploring the biological and socio-economic potential of new/emerging fish species for the expansion of the European aquaculture industry".

References

Andree K.B., Roque A., Duncan N., Gisbert E., Estevez A., Tsertou M.I., Katharios P. 2015. *Diplectanum sciaenae* (Van Beneden & Hesse, 1863) (Monogenea) infecting meagre, *Argyrosomus regius* (Asso, 1801) broodstock in Catalonia, Spain. A case report. Veterinary Parasitology: Regional Studies and Reports 1–2, 75-79.

Andreoni S., Farina C., Lombardi G. 2003. Atlante di micologia medica. Ed. Systems, Vigano di Gaggiano (MI).

Aspehaug V., Devold M., Nylund A. 1999. The phylogenetic relationship of nervous necrosis virus from halibut (*Hippoglossus hippoglossus*). Bull. Eur. Assoc. Fish Pathol. 19, 196-202.

Avsever M.L., Cavusoglu C., Gunen M.Z., Yazicioglu O., Eskiizmirliler S., Didinen B.I., Tunaligil S., Erdal G., Ozden M. 2014. The first report of *Mycobacterium marinum* isolated from cultured meagre, *Argyrosomus regius*. Bull. Europ. Ass. Fish Pathol. 34, 124-129.

Baily J.E., Bretherton M.J., Gavine F.M., Ferguson H.W., Turnbull J.F. 2005. The pathology of chronic erosive dermatopathy in Murray cod *Maccullochella peelii peelii.* J. Fish Dis. 28, 3-12.

Barnes A.C., dos Santos N.M.S., Ellis A.E. 2005. Progress in fish vaccinology. 3rd International Symposium, Bergen, April 2003. Proceedings ISBN: 978-3-8055-7936. In: Midtlyng, P.J. (ed) (Oslo) Society: International Alliance for Biological Standardization (IABS), 121, 75-84.

Bottari T., Foti M., Marino F., Mancuso M., Manfrin A. 2009. Susceptibility of some new fish species to *Photobacterium damselae* subsp. *piscicida* in challenge. Ittiopatologia 6, 17-21.

Bovo G., Nishizawa T., Maltese C., Borghesan F., Mutinelli F., Montesi F., De Mas S. 1999. Viral encephalopathy and retinopathy of farmed marine fish species in Italy. Virus Res 63, 143-146.

Cardeira J., Vallés R., Dionísio G., Estévez A., Gisbert E., Pousão-Ferreira P., Cancela M. L., Gavaia P. J. 2012. Osteology of the axial and appendicular skeletons of the meagre, *Argyrosomus regius* (Teleostei, Sciaenidae) and early skeletal development at two rearing facilities. J. Appl. Ichthyol. 28, 464-470.

Cardenas S. 2011. Cultivo de corvina (*Argyrosomus regius*). Publicación Madrid, Fundación Observatorio Español de Acuicultura, Consejo Superior de Investigaciones Científicas. 96 p. ISBN: 978-84-00-09291-7

Castanho S., Gavaia P.J., Pinto W., Candeias-Mendes A., Pousão-Ferreira P., Conceição L., Dias J. 2017. Dietary mineral and vitamin premixes affect growth and skeletal quality of meagre *Argyrosomus regius* post-larvae. Aquaculture Europe 2017 Cooperation for growth. October 17-20, Dubrovnik, Croatia.

Ciulli S., Galletti E., Grodzki M., Alessi A., Battilani M., Prosperi S. 2007. Isolation and genetic characterization of betanodavirus from wild marine fish from the Adriatic Sea. Vet. Res. Comm. 31, 221–224.

Colorni A. 2004. Diseases of mediterranean fish species: Problems, research and prospects. Bull. Europ. Ass. Fish Pathol. 24, 22-32.

Cutrin J.M., Dopazo C.P., Thiery R., Leao P., Olveira J.G., Barja J.L., Bandin I. 2007. Emergence of pathogenic betanodaviruses belonging to the SJNNV genogroup in farmed fish species from the Iberian Peninsula. J. Fish Dis. 30, 225–232.

Duncan N., Estevez A., Fernández-Palacios H., Vallés R. 2013. Aquaculture production of meagre (*Argyrosomus regius*): Hatchery techniques, ongrowing and market. In book: Advances in aquaculture hatchery technology, Chapter: 17, Publisher: Woodhead Publishing, Editors: Geoff Allan and Gavin Burnell, pp.519-541.

Duncan N., Estevez A., Padros F., Aguilera C., Montero F.E., Norambuena F., Carazo I., Carbo R., Mylonas C. 2008. Acclimation to captivity and GnRHa-induced spawning of meagre (*Argyrosomus regius*). Cybium 32 suppl., 332-333.

Elkesh A., Kantham K.P.L., Shinn A.P., Crumlish M., Richards R.H. 2013. Systemic nocardiosis in a Mediterranean population of cultured meagre, *Argyrosomus regius* Asso (Perciformes: Sciaenidae). J. Fish Dis. 36(2), 141-149.

Fanouraki E., Mylonas C.C., Papandroulakis N., Pavlidis M. 2011. Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. Gen. Comp. Endocrinol. 173, 313-322.

García A. B., Benavides J., Campos P., Sánchez-Mut A., Lozano E., Alcázar M. C, Amaya J.F., Isern M.M. 2013. Vibriosis in west mediterranean facilities. Workshop: Vibriosis in aquaculture. 4 September 2013, Finland. European Association of Fish Pathologists. Available from: http://eafp.org/s/eafp-2013-workshop-vibriosis-in-aquaculture-m-isern.

Gavaia P. J., Dinis M. T., Cancela M. L. 2002. Osteological development and abnormalities of the vertebral column and caudal skeleton in larval and juvenile stages of hatchery-reared Senegal sole (*Solea senegalensis*). Aquaculture 211, 305-323.

Georgakopoulou E., Katharios P., Divanach P., Koumoundouros G. 2010. Effect of temperature on the development of skeletal deformities in Gilthead seabream (*Sparus aurata* Linnaeus, 1758). Aquaculture 308, 13-19.

Ghittino C., Manuali E., Latini M., Agnetti F., Rogato F., Agonigi R., Colussi S., Prearo M. 2004. Case of systemic granulomatosis in meagre (*Argyrosomus regius*) and comparison with the histological features present in gilthead seabream. Ittiopatologia 1, 59-67.

Haenen O. L. M., Fouz B., Amaro C., Isern M. M., Mikkelsen H., Zrncic S., Travers M. A., Renault T., Wardle R., Hellström A., Dalsgaard I. 2013. Vibriosis in aquaculture. 16 th EAFP Conference Tampere, Finland, 4th September 2013. Workshop Report. Bull. Europ. Ass. Fish Pathol. 34, 138-148.

Hodneland K., Garcia R., Balbuena J. A., Zarza C., Fouz B. 2011. Real-time RT-PCR detection of betanodavirus in naturally and experimentally infected fish from Spain. J. Fish Dis. 34, 189-202.

Katharios P., Kokkari K., Papadaki M., Papandroulakis N. 2011a. Systemic granulomas in cultured meagre, *Argyrosomus regius*. In: Aquaculture Europe 11, Rhodes, pp. 537-538.

Katharios P., Papadaki M., Ternengo S., Kantham P. K., Zeri C., Petraki P. E. 2011b. Chronic ulcerative dermatopathy in cultured marine fishes. Comparative study in sharpsnout sea bream, *Diplodus puntazzo* (Walbaum). J. Fish Dis. 34(6), 459-74.

Kent M. L. 2000. Marine netpen farming leads to infections with some unusual parasites. Int. J. Parasitol. 30, 321-326.

Kir M., Topuz H., Sunar M. C., Topuz M. 2015a. Effect of temperature on acute toxicity of nitrite to meagre *Argyrosomus regius* (Asso, 1801). J. World Aquac. Soc. 46, 564-568.

Kir M., Topuz H., Sunar M. C., Topuz M. 2015b. Acute toxicity of ammonia in Meagre (*Argyrosomus regius* Asso, 1801) at different temperatures. Aquac. Res. 1-6. doi:10.1111/are.12811

Koumoundouros G., Maingot E., Divanach P., Kentouri M. 2002. Kyphosis in reared sea bass (*Dicentrarchus labrax* L.): ontogeny and effects on mortality. Aquaculture 209, 49-58.

Kružić N., Mustać B., Župan I., Čolak S. 2016. Meagre (*Argyrosomus regius* Asso, 1801) aquaculture in Croatia. Croatian Journal of Fisheries, 14-19.

Labella A., Berbel C., Manchado M., Castro D., Borrego J. J. 2011. *Photobacterium damselae* subsp. *damselae*, an emerging pathogen affecting new cultured marine fish species in southern Spain, in *Recent Advances in Fish Farms* eds Aral Faruk, Doğu Zafer., editors. (New York: InTech), 135-152.

Labella A., Manchado M., Alonso M. C., Castro D., Romalde J. L., Borrego J. J. 2010. Molecular intraspecific characterization of *Photobacterium damselae* ssp. *damselae* strains affecting cultured marine fish. J. Appl. Microbiol. 108, 2122-2132.

Labella A., Vida M., Alonso M.C., Infante C., Cardenas S., López-Romalde S., et al. 2006. First isolation of *Photobacterium damselae* ssp. *damselae* from cultured redbanded seabream, *Pagrus auriga* Valenciennes, in Spain. J. Fish Dis. 29, 175-179.

Le Breton A., Grisez L., Sweetman J., Ollevier F. 1997. Viral nervous necrosis (VNN) associated with mass mortalities in cage-reared sea bass, *Dicentrarchus labrax* (L.). J. Fish Dis. 20, 145-151.

Le Vay L., Carvalho G. R., Quinitio E. T., Lebata J. H., Fushimi V. N., Ut H. 2007. Quality of hatchery-reared juveniles for marine fisheries stock enhancement. Aquaculture 268, 169-180.

Leong T. S., Colorni A. 2002. Infectious diseases of warmwater fish in marine and brackish waters. In: Woo PTK, Bruno W, Lim SLH (eds) Diseases and disorders of finfish in cage culture. CAB International, Wallingford, p. 193-230.

Lopez-Jimena B., Cherif N., Garcia-Rosado E., Infante C., Cano I., Castro D., Hammami S., Borrego J. J., Alonso M. C. 2010. A combined RT-PCR and dot-blot hybridization method reveals the coexistence of SJNNV and RGNNV betanodavirus genotypes in wild meagre (*Argyrosomus regius*). J. Appl. Microbiol. 109, 1361-1369.

Martins G., Pousão-Ferreira P., Gavaia P. J. 2013. Evaluation of skeletal deformities in the meagre reared under different nutritional conditions. 3rd Interdisciplinary Approaches in Fish Skeletal Biology. Tavira, Portugal 22-24 April 2013.

Manuali E., Agnetti F., Latini M., Checcarelli S., Ghittino C. 2005. Outbreak of systemic mycosis in intensively reared meagre *(Argyrosomus regius).* Ittiopatologia 2, 129-135.

Mendes A. M., Moreno A. 2001. A case of a fracture of the rachis in a corvine (*Argyrosomus regius*, Asso, 1801). Rev. Port Cienc. Vet. 96, 161-162.

Merella P., Cherchi S., Garippa G., Fioravanti M.L., Gustinelli A., Salati F. 2009. Outbreak of *Sciaenacotyle panceri* (Monogenea) on cage-reared meagre *Argyrosomus regius* (Osteichthyes) from the western Mediterranean Sea. Dis. Aquat. Organ. 86, 169-173.

Munday B., Kwang J., Moody N. 2002. Betanodavirus infections of teleost fish: a review. J. Fish Dis. 25, 127-142.

Nishizawa T., Furuhashi M., Nagai T., Nakai T., Muroga K. 1997. Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. Appl. Environ. Microbiol. 63, 1633-1636.

Nylund A., Karlsbakk E., Nylund S., Isaksen T. E., Karlsen M., Korsnes K., Handeland S., Martinsen R., Mork Pedersen T., Ottem K. F. 2008. New clade of betanodaviruses detected in wild and farmed cod (*Gadus morhua*) in Norway. Arch. Virol. 153, 541-547.

Office International des Epizooties, 2003. Viral encephalopathy and retinopathy. In: Manual of Diagnostic Tests for Aquatic Animals. OIE, Paris, pp. 135-14.

Oliver G. 1980. Les Diplectanidae Bychowsky 1957 (Monogenea, Monopisthocotylea) parasites des Scianenidae (Pices, Perciformes) du Golfe de Gascogne. Bull. Mus. Natl. Hist. Nat., 2, 669-689.

Olveira J. G., Soares F., Engrola S., Dopazo C. P., Bandin I. 2008. Antemortem versus postmortem methods for detection of betanodavirus in Senegalese sole (*Solea senegalensis*). J. Vet. Diag. Invest. 20, 215-219.

Olveira J. G., Douto S., Dopazo C. P., Thiery R., Barja J. L. 2009. Comparative analysis of both genomic segments of betanodaviruses isolated from epizootic outbreaks in farmed fish species provides evidence for genetic reassortment. J. Gen. Virol. 90, 2940-2951.

Quilichini Y., Foata J., Marchand B. 2009. Ultrastructural study of spermiogenesis and the spermatozoon of *Microcotyle pancerii* (monogenea: polyopisthocotylea: microcotylidae), parasite of meagre *Argyrosomus regius* (Pisces: teleostei). J. Parasitol. 95, 261-270.

Rigos G., Katharios P. 2010. Pathological obstacles of newly-introduced fish species in Mediterranean mariculture: a review. Rev.Fish Biol. Fisher. 20, 47-70.

Saad T. T. 2013. Some Study on Heavy Metal and Bacterial infection Relationship in Some Cultured Marine Fish. J. arab. Aquac. Soc. 8, 143-162.

Schultz A. G., Shigdar S. L., Jones P. L., Ward A. C., Toop T. 2011. Groundwater pre-treatment prevents the onset of chronic ulcerative dermatopathy in juvenile Murray cod, *Maccullochella peelii peelii* (Mitchell). Aquaculture 312, 19-25.

Skliris G. P., Krondiris J. V., Sideris D. C., Shinn A. P., Starkey W. G., Richards R. H. 2001. Phylogenetic and antigenic characterization of new fish nodavirus isolates from Europe and Asia. Virus Res. 75, 59-67.

Soares F., Monzón I., Costas B., Gavaia P. 2014. Non-infectious Disorders of Warmwater Fish. Chapter 7. In: "Diseases and Disorders of Finfish in Cage Culture, 2nd edition". P. Woo, D. Bruno, Eds. CRC Press (Taylor and Francis), 2014. ISBN: 9781780642079. 254-286.

Soares F., Quental-Ferreira H., Moreira M., Mendes A. C., Gamboa M., Barata M., Ribeiro L., Cunha M. E., Pousão-Ferreira P. 2012[a]. Pathologies affecting farmed meagre (*Argyrosomus regius*) at Ipimar's research center - southern Portugal. Aqua 2012. Prague, Czech Republic, 1-5 september 2012.

Soares F., Quental Ferreira H., Moreira M., Cunha E., Ribeiro L., Pousão-Ferreira P. 2012b. First report of *Amyloodinium ocellatum* in farmed meagre (*Argyrosomus regius*). Bull. Europ. Ass. Fish Pathol. 32(1), 30-33.

Soares F., Leitão A., Moreira M., Teixeira de Sousa J., Almeida A.C., Barata M., Feist S. W., Pousão-Ferreira P., Ribeiro L. 2012c. Presence of sarcoma in the thymus of meagre juveniles (*Argyrosomus regius*, Asso) reared in an intensive system. Dis. Aquat. Organ. 102, 119-127.

Soares F., Ribeiro L., Barata M., Pousão-Ferreira P. 2011. Sarcoma in the thymus of meagre juveniles (*Argyrosomus regius*). Bull. Europ. Ass. Fish Pathol. 31, 189.

Ternengo S., Agostini S., Quilichini Y., Euzet L., Marchand B. 2010. Intensive infestations of *Sciaenocotyle pancerii* (Monogenea, Microcotylidae) on *Argyrosomus regius* (Asso) under fish-farming conditions. J. Fish Dis. 33, 89-92.

Toksen E., Buchmann K., Bresciani J. 2007. Occurrence of *Benedenia sciaenae* van Beneden, 1856 (Monogenea: Capsalidae) in cultured meagre (*Argyrosomus regius* Asso, 1801) (Teleost: Sciaenidae) from western Turkey. Bull. Europ. Ass. Fish Pathol. 27, 250-253.

Witten P. E., Obach A., Huysseune A., Baeverfjord G. 2006. Vertebrae fusion in Atlantic salmon (*Salmo salar*): development, aggravation and pathways of containment. Aquaculture 258, 164-172.

Figure legends

Figure 1. Vertebral column organization *Argyrosomus regius*. The scheme represents the typical distribution of vertebrae in juveniles and adults with 5 cephalic (C), 5 pre haemal (PH), 12 haemal (H) and 3 caudal (Cf) vertebrae that include the urostile (U). See text for details.

Figure 2. Skeletal deformities affecting the vertebral column of juveniles and young adults of *Argyrosomus regius*. A- Adult with normal skeletal phenotype; B- Adult fish displaying a severe lordotic curvature in the haemal vertebrae caused by fusion and deformation of vertebrae 12 -14. C- Juvenile with normal skeletal phenotype. D- Juvenile displaying vertebral fusions in vertebrae 9-10 and 12-13 (arrowheads) and deformed neural arches and spines (arrows).

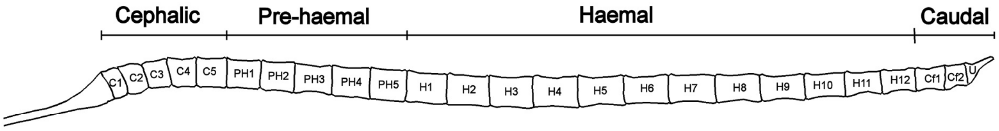

Figure 1. Vertebral column organization Argyrosomus regius. The scheme represents the typical distribution of vertebrae in juveniles and adults with 5 cephalic (C), 5 pre haemal (PH), 12 haemal (H) and 3 caudal (Cf) vertebrae that include the urostile (U). See text for details.

659x226mm (72 x 72 DPI)

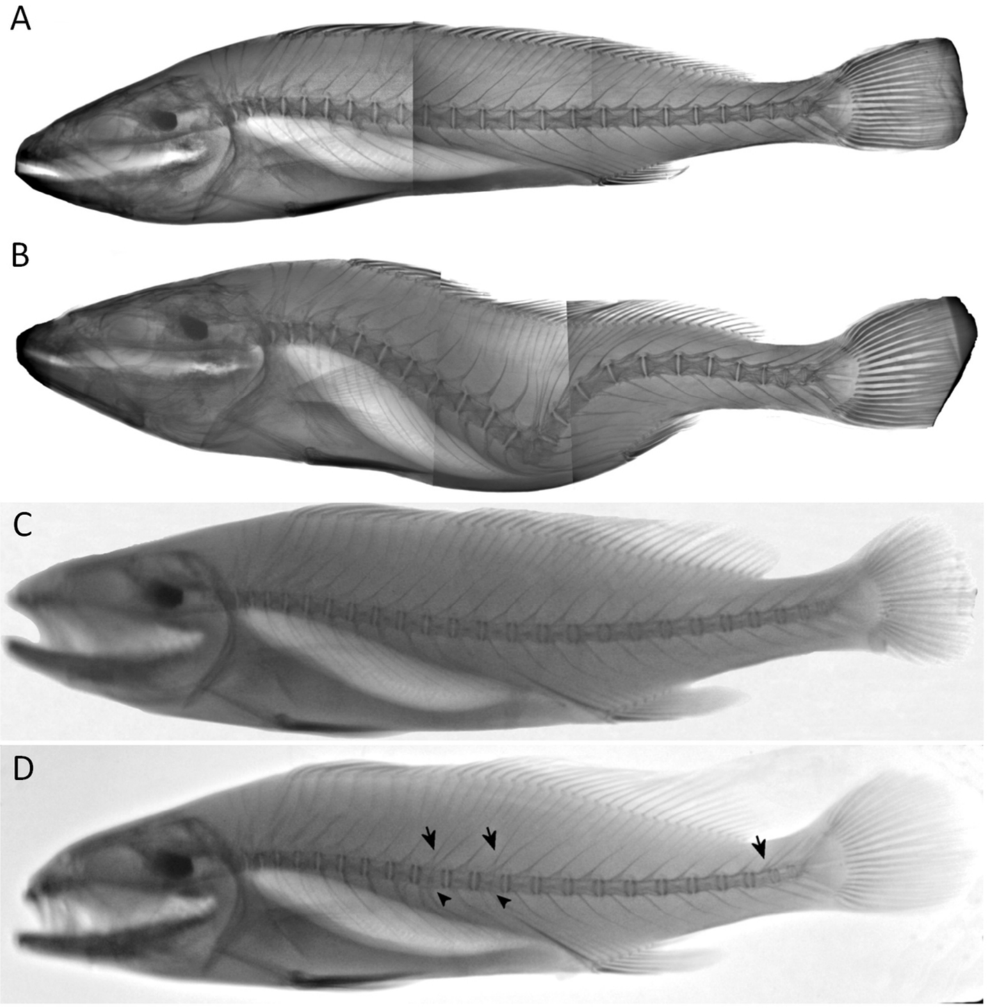

Figure 2. Skeletal deformities affecting the vertebral column of juveniles and young adults of Argyrosomus regius. A- Adult with normal skeletal phenotype; B- Adult fish displaying a severe lordotic curvature in the haemal vertebrae caused by fusion and deformation of vertebrae 12 -14. C- Juvenile with normal skeletal phenotype. D- Juvenile displaying vertebral fusions in vertebrae 9-10 and 12-13 (arrowheads) and deformed neural arches and spines (arrows).

108x111mm (300 x 300 DPI)

Diseases caused by:	***Species***	**Production system**	**Reference**
PARASITES			
Monogenea, Microcotylidae	*Sciaenocotyle pancerii*	Cages (Corsica - France) Cages (Sardinia-Italy)	Ternengo et al. 2010 Merela et al. 2009
Monogenea: Microcotylidae	*Microcotyle pancerii*	(Corsica-France)	Quilichini et al. 2009
Monogenea: Capsalidae	*Benedenia sciaenae*	Cages (Turkey)	Toksen et al. (2007)
Monogenea: Monopisthocotylea	*Diplectanum sciaenae*	Tanks (Spain)	Andree et al. (2016)
Dinoflagellate	*Amyloodinium ocellatum*	Ponds – Semi-intensive (Portugal)	Soares et al. (2012b)
Trematoda	*Calceostoma* spp.	20 m^3 tanks (Spain)	Duncan et al. (2008)
Trematoda	*Sciaenocotyle* spp.	Cages (Corsica)	Ternengo et al. (2010)
FUNGOS			
Fungal colonies	*Penicillium digitatum*	Intensive	Manuali et al. (2005)
BACTERIA			
Vibrioses	*Vibrio tapetis*	Centro IFAPA, El Toruño (Spain)	Cardenas (2011)
Pasteurelosis	*Photobacterium damselae subsp. piscicida*	Challange test Cultured	Bottari et al. (2009) Labella et al. (2006, 2010, 2011)
Nocardia	Nocardia-*like*	Cages (Greece)	Elkesh et al. (2013)
NON INFECTIOUS DISEASES			
Granulomatosis			Ghittino et al. (2004)
Thymus Sarcoma		Intensive (Portugal)	Soares et al. (2012b,c)
Chronic erosive dermatopathy			Katharios et al. (2011)
Lordosis-kyphosis		Ponds – Semi intensive (Portugal)	Soares et al. (2014)
Vertebral fusions		Intensive (Portugal)	Martins et al. (2013)

Skeleton	Structures		Mode	Range
Axial	Vertebrae	Cephalic	5	4-5
		Pre-haemal	5	5-6
		Haemal	11	10-12
		Caudal	2	2
		Urostile	1	1
		Total	25	23-26
	Epipleural ribs		9	8-10
	Pleural ribs		9	7-9
Appendicular	Dorsal fin	Spines	XI	X-XI
		Lepidotrichia	27	25-30
		Pterygiophores	35	33-37
		Pre-dorsals	3	3
	Anal fin	Spines	II	II
		Lepidotrichia	8	7-8
		Pterygiophores	8	7-8
	Caudal fin	Dermatotrichia	11	10-12
		Lepidotrichia	20	19-22
		Hypurals	6	6
		Epurals	2	1-3