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Increased melanomacrophage centres in the liver of reproductively dysfunctional female greater amberjack *Seriola dumerili* (Risso 1810)

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Abstract

The greater amberjack *Seriola dumerili* is a new aquaculture fish that may display reproductive dysfunctions. During extensive follicular atresia, which is a common reproductive dysfunction in females during vitellogenesis, part of the reabsorbed yolk returns to the liver to be metabolised and recycled. Melanomacrophage centres (MMCs) are aggregates of macrophage-like cells that play a role in the destruction, detoxification, and recycling of endogenous and exogenous materials, and have been associated with systemic stress. Wild and captive-reared greater amberjack were sampled in the Mediterranean Sea during two different phases of the reproductive cycle. The liver of reproductively dysfunctional captive-reared females sampled during the spawning season, showed a high density of both MMCs and apoptotic cells. A weak liver anti-cytochrome P450 monooxygenase 1A immunoreactivity was observed, suggesting that the examined fish were not exposed to environmental pollutants. We propose that the observed increase of MMCs and apoptosis in captive-reared fish was related to the hepatic overload associated to the metabolism of yolk proteins reabsorbed during extensive follicular atresia. Since follicular atresia is a frequent physiological and pathological event in teleosts, we suggest that the reproductive state should be always assessed when MMCs are used as markers of exposure to stress or pollutants.

Keywords: fish liver; spawning omission; reproductive dysfunction; follicular atresia; apoptosis.

Running Head: Melanomacrophage centres in greater amberjack liver.

List of abbreviations:

AC_d	Density of apoptotic cells
AF_f	Fraction of atretic follicles
AF_p	Percentage of atretic follicles
GSI	Gonado-somatic index
HSI	Hepato-somatic index
MMCs	Melanomacrophage centres
MMC_d	Density of melanomacrophage centres
MMC_n	Number of melanomacrophage centres
MMC_{sz}	Size of melanomacrophage centres

1 INTRODUCTION

The greater amberjack *Seriola dumerili* (Risso 1810) is an epibenthic and pelagic teleost species distributed in the Indo-West Pacific (Paxton, Hoese, Allen, & Hanley, 1989), western Atlantic (Cervigón, 1993; Smith, 1997) and eastern Atlantic oceans as well as in the Mediterranean Sea (Bauchot, 1987). Thanks to its rapid growth rate, high flesh quality and consumers' appreciation, the greater amberjack is a promising new aquaculture species (Fakriadis, Lisi, Sigelaki, Papadaki, & Mylonas, 2019).

Recent studies conducted in the Mediterranean Sea showed that the greater amberjack, as many other fishes, exhibits reproductive dysfunctions when caught from the wild and confined in captivity (Zupa, Fauvel, Mylonas, Pousis, Santamaria, Papadaki, Fakriadis, Cicirelli, Mangano, Passantino, Lacalandra, & Corriero, 2017a; Zupa, Rodríguez, Mylonas, Rosenfeld, Fakriadis, Papadaki, Pérez,

Pousis, Basilone, & Corriero, 2017b; Pousis, Mylonas, De Virgilio, Gadaleta, Santamaria, Passantino, Zupa, Papadaki, Fakriadis, Ferreri, & Corriero 2018; Pousis, Rodríguez, De Ruvo, De Virgilio, Pérez, Mylonas, Zupa, Passantino, Santamaria, Valentini, & Corriero, 2019). Captive-reared greater amberjack females sampled during the reproductive season displayed an extensive atresia of advanced vitellogenic oocytes (Zupa et al., 2017b, Pousis et al., 2018), which was possibly caused by stress during handling operations in the rearing cages (Zupa et al., 2017a, b; Pousis et al., 2018, 2019). Atretic degeneration of ovarian follicles, as well as apoptosis of germ cells in males, are normal physiological events of vertebrate gonadal morphogenesis that seem to be essential for the regulation of the energetic homeostasis and for the selection of germ cells more suitable for the production of viable embryos (Corriero, Desantis, Bridges, Kime, Megalofonou, Santamaria, Cirillo, Ventriglia, Di Summa, Deflorio, Campobasso, & De Metrio, 2007; Krysko, Diez-Fraile, Criel, Svistunov, Vandenabeele, & D'Herde, 2008). However, extensive follicular atresia during vitellogenesis may occur after severe stress in reared fish (Corriero, Zupa, Bello, Mylonas, Deflorio, Genovese, Basilone, Buscaino, Buffa, Pousis, De Metrio, & Santamaria, 2011), and leads to failure to undergo oocyte maturation, and thus ovulation and spawning.

In teleost fishes, follicular atresia is involved in normal ovarian growth and postovulatory regression, mostly in females that are not able to carry out maturation or ovulation after vitellogenesis (Agulleiro, André, Morais, Cerdà, & Babin, 2007). Therefore, follicular atresia, similar to male germ cell apoptosis (Corriero et al., 2007), plays an important role in the control of gamete production. Moreover, follicular atresia is one of the mechanisms leading variable fractions of wild fish populations to skip reproduction (Jørgensen, Ernande, Fiksen, & Dieckmann, 2006). Several factors influence follicular atresia, including temperature, starvation and stress (Guraya, 1986; Krysko et al., 2008), and can affect both vitellogenic and previtellogenic follicles (Hunter & Macewicz, 1985; Guraya, 1986; Rizzo & Bazzoli, 1995; Miranda, Bazzoli, Rizzo, & Sato, 1999). The complex mechanisms leading to ovarian follicle atresia are not fully elucidated and seem to involve autophagy, heterophagy and apoptosis (Santos, Thomé, Arantes, Sato, Bazzoli, & Rizzo, 2008). Little is known

about the fate of the oocyte components reabsorbed during this process; however, there is evidence that part of the yolk proteins is degraded into free amino acids (Wood &Van Der Kraak, 2003) and part of the highly energetic moieties derived by phagocytosed yolk return to the liver through the blood to be further metabolised and recycled (Babin, 1987a, b).

Melanomacrophage centres (MMCs) are aggregates of macrophage-like cells, which are fragments derived from phagocytosed cells and pigments, such as melanin, haemosiderin, and lipofuscin (Roberts, 1975; Fournie, Summers, Courtney, & Engle, 2001; Agius & Roberts, 2003), located in the reticuloendothelial tissue of haemolymphopoietic organs (liver included) of various non-mammalian vertebrates (Wolke, 1992; Christiansen, Grzybowski, & Kodama, 1996; Rund, Christiansen, & Johnson, 1998; Fournie et al., 2001; Barni, Vaccarone, Bertone, Fraschini, Bernini, & Fenoglio, 2002; Loumbourdis & Vogiatzis, 2002; Koppang, Haugarvoll, Hordvik, Aune, & Poppe, 2005; Fishelson, 2006). Melanomacrophage centres play a role in the destruction, detoxification, and recycling of endogenous and exogenous materials, including dead cells and cell debris (Agius & Roberts, 1981; van der Oost, Beyer, & Vermeulen, 2003; Mela, Randi, Ventura, Carvalho, Pelletier, & Oliveira Ribeiro, 2007). An increase in liver MMC density has been also associated with cytochrome P450 monooxygenase 1A (CYP1A) expression (van der Weiden, Bleumink, Seinen, & van den Berg, 1994; Passantino, Santamaria, Zupa, Pousis, Garofalo, Cianciotta, Jirillo, Acone, & Corriero, 2014; Basilone, Gargano, Corriero, Zupa, Santamaria, Mangano, Ferreri, Pulizzi, Mazzola, Bonanno, & Passantino, 2018) and apoptotic cell death (Gogal, Smith, Robertson, Smith, & Holladay, 1999; Corriero, Zupa, Pousis, Santamaria, Bello, Jirillo, Carrassi, De Giorgi, & Passantino, 2013) after fish exposure to toxic compounds. Hence, MMCs are useful response (effect) biomarkers of exposure to environmental pollutants (Agius, 1979; Agius & Roberts, 1981, 2003; Fishelson, 2006; Passantino et al., 2014). Moreover, changes in the MMC characteristics of several cultured fish species as a result of dietary manipulations (Phromkunthong, Nuntapong, Wanlem, & Boonyaratpalin, 2015), exposure to infectious agents (Zhang, Li, Mo, Luo, Sun, Liu, Li, Zhou, & Dan, 2014; Yunis-Aguinaga,

Claudiani, Marcusso, Manrique, Engrácia de Moraes, de Moraes, & Fernandes, 2015) and mere captivity-induced stress (Evans & Norvak, 2016) have been documented.

The present study examined the occurrence of MMCs in the liver of reproductively dysfunctional captive-reared greater amberjack, in comparison to naturally maturing fish in the wild, in order to increase our knowledge on the role of MMCs, as well as on their reliability as marker of stress and exposure to environmental pollutants.

2 MATERIALS AND METHODS

2.1 Ethics statement

The present study was carried out using liver samples taken from captive and wild fish coming from a registered aquaculture facility and commercial catches, respectively (see § 2.2). Ethical approval was not required because this study did not involve any experiments on alive animals.

The greater amberjack is classified as "Least Concern" in the IUCN Red List of Threatened Species (Smith-Vaniz, Pina Amargos, Brown, Curtis, & Williams, 2015).

2.2 Sample collection

For the present study 25 (11 males and 14 females) liver samples taken from wild greater amberjack and 22 (10 males and 12 females) liver samples taken from captive-reared greater amberjack were used. The samples were part of a larger sampling carried out during the reproductive season 2014 and 2015 in the framework of a study on the effects of rearing in captivity on gametogenesis and reproduction (Zupa et al., 2017a, b; Pousis et al. 2018, 2019).

Wild individuals were captured around the Pelagie Islands (Sicily, Italy) by the commercial purse seine fishing vessel "Graziella" authorized to catch pelagic fish by the port authority of Porto Empedocle (Agrigento, Italy). No specific permission was required because these fish were commercially caught during routine fishing operations, placed on ice by the fishermen and left to die.

158 Immediately after death, those fish considered suitable for the study were purchased and sampled on
159 board.
160 Captive-reared fish originally came from the fishery at 0+ year of age, and were then reared at a
161 registered aquaculture facility for 3 years, according to routine farming practices. These individuals
162 were captured from the wild in 2011 in the area of Astakos (Ionian Sea, Greece) and, in September
163 2014, they were transferred to a sea cage of Argosaronikos Fishfarming S.A. (Salamina Island,
164 Greece), where they were reared for two years according to standard farming practices. The fish were
165 fed to apparent satiation every other day, during the first year fresh fish, while during the year of the
166 sampling the fish were switched to a commercial extruded broodstock diet (Vitalis-Cal, Skretting SA,
167 Norway) as it is customary for aquaculture breeders of many species. Before sampling, captive-reared
168 fish were confined in a small cage area using a PVC curtain and then were tranquilized with about
169 0.01 ml l-1 clove oil (Roumpoulakis E.P.E., Greece) dissolved in ethanol at a 1:10 ratio (Mylonas,
170 Cardinaletti, Sigelaki, & Polzonetti-Magni, 2005). Then, they were gently directed into a PVC
171 stretcher, brought on board of a service vessel, anesthetized deeply with 0.03 ml l-1 clove oil and
172 finally euthanized by decapitation (Metcalf & Craig, 2011).
173 From each fish, sampling date, fork length (FL, cm) and body mass (BM, kg) were recorded
174 (Tables 1 and 2; Supplementary File 1), and gonads and livers were dissected and weighted
175 (Supplementary file 1). Liver samples (about 0.5 x 0.5 x 0.5 cm) were taken in proximity of the hilum
176 (Figure 1) and fixed in 10% buffered formalin. From females, one-cm- thick gonad samples were
177 taken and fixed in Bouin's solution for the calculation of the percentage of atretic follicles. Gonado-
178 somatic index was calculated as GSI = 100 Gonad Mass/Body Mass; hepato-somatic index was
179 calculated as HSI = 100 Liver Mass/Body Mass (Tables 1 and 2; Supplementary file 1).
180 The reproductive state of the sampled fish was described by Zupa et al. (2017a, b). Briefly, the
181 fish (both wild and captive-reared) sampled during late April-early June were in active
182 gametogenesis, since males showed all stages of spermatogenesis and females had oocytes in early
183 and/or late vitellogenesis. Wild fish sampled in late June-July were in active spawning, showing testes

with seminiferous lobules filled with spermatozoa and ovaries containing oocytes in final maturation and/or post-ovulatory follicles, whereas captive-reared fish sampled in the same period showed a severe reproductive dysfunction, characterized by arrested spermatogenesis and extensive atresia of vitellogenic follicles.

In order to analyse the changes in the amount of MMCs according to the sex, sampling period and condition (wild *vs* captive-reared), the sampled fish were subdivided in the following groups (Tables 1 and 2): i. wild males sampled during gametogenesis; ii. wild males sampled during spawning; iii. captive-reared males sampled during gametogenesis; iv. captive-reared males sampled during spawning; v. wild females sampled during gametogenesis; vi. wild females sampled during spawning; vii. captive-reared females sampled during gametogenesis and viii. captive-reared females sampled during spawning.

2.3 Histology, histochemistry and apoptosis

Gonad and liver samples were embedded in paraffin wax after dehydration in increasing ethanol concentrations and clarification in xylene. For the description of the liver structure, five to ten liver sections (5 µm thick) were cut and stained with Mallory's trichrome. Moreover, Mallory's fuchsin (Mazzi, 1977) and Perls-Van Gieson stainings were used to identify lipofuscin–ceroids and ferric ions of hemosiderin, respectively.

The immunohistochemical detection of CYP1A was performed on liver sections of all the sampled fish according to the procedure by Passantino et al. (2014) and Basilone et al. (2018). Briefly, liver sections were deparaffinized in xylene, rehydrated through graded ethanol solutions, pre-treated for 10 min with 3% H_2O_2 to inhibit endogenous peroxidase activity. Non-specific binding sites for immunoglobulins were blocked by incubation for 30 min in normal horse serum (NHS), and sections were then incubated for 60 min at 37 °C with polyclonal anti-fish CYP1A peptide (Biosense Laboratories, Bergen, Norway). The antibody was diluted 1:50; 1:100 and 1:500 in PBS containing 0.1% bovine serum albumin (BSA). Liver sections of anchovies sampled in a marine area exposed to

pollutants of industrial/agricultural origin (Basilone et al., 2018) were used as positive control. The immuno-histochemical visualization was obtained using the Vectastain Universal Elite Kit (Vector, Burlingame, CA).

The detection of liver apoptotic cells was performed on de-paraffinized sections of all the wild and captive-reared fish sampled during the spawning period by the terminal deoxynucleotidyl transferase mediated d'UTP nick end labelling (TUNEL) method, using an *In Situ Cell Death Detection Kit, AP* (Roche Diagnostics, Mannheim, Germany) (Corriero et al., 2007). Prior to incubation with the reaction mixture, the sections, after their re-hydration through graded ethanol solutions, were incubated in a permeabilization solution of 0.1% Triton X-100 in 0.1% sodium citrate for 8 min at 37 °C. Terminal deoxynucleotidyl transferase was diluted 1:20 in TUNEL Dilution Buffer (Roche Diagnostics, Mannheim, Germany). A ready to use solution of nitro blue tetrazolium chloride/5-bromo-4chloro-3indolyl phosphate, toluidine salt (NBT/BCIP) (Roche Diagnostics, Mannheim, Germany) was used as a substrate for the signal conversion.

The identification of atretic vitellogenic follicles was made on haematoxylin-eosin stained ovary sections on the basis of the description reported by Hunter & Macewicz (1985) and Corriero, Acone, Desantis, Zubani, Deflorio, Ventriglia, Bridges, Labate, Palmieri, McAllister, Kime, & De Metrio, (2004). Briefly, vitellogenic follicles in α atresia are characterized by the disintegration of the oocyte nucleus and cytoplasmic organelles, followed by zona radiata (chorion) fragmentation. The following β atresia stage is characterized by complete reabsorption of zona radiata and yolk globules.

2.4 Relative quantification of MMCs, apoptotic cells and atretic vitellogenic follicles

The density of MMCs (MMC_d; μm^2 MMCs/mm^2 hepatic parenchyma), their mean size (MMC_{sz}; μm^2) and their number per mm^2 hepatic parenchyma (MMC_n) were measured on liver sections stained by Mallory's trichrome and photographed with a 40x objective with a digital camera (DFC 420, Leica) connected to a light microscope (DIAPLAN, Leitz) (digital fields surface area = 95 600 μm^2). Measurements were taken using an image analysis software (Leica Application Suite, version 3.3.0).

The density of apoptotic cells (AC_d; number of TUNEL-positive cells/mm^2 hepatic parenchyma) was measured on liver sections using the same procedure and equipment above reported for MMCs.

The fraction and the percentage of atretic follicles were calculated in females whose ovaries showed oocytes in late vitellogenesis stage. For each ovary, digital fields were photographed randomly with a 10x objective using a digital camera (DFC 420, Leica) connected to a light microscope (DIAPLAN, Leitz). For each digital field, all the ovarian follicles in late vitellogenesis, including α and β atretic follicles were identified and counted. The fraction (AF_f) of atretic follicles was calculated as the number of atretic follicles/total number of late vitellogenic follicles; the percentage of atretic follicles (AF_p) was calculated as 100 AF_f.

2.5 Statistical analyses

Normal distribution of GSI, HIS, MMC_d, MMC_{sz}, MMC_n and AC_d was confirmed through the Shapiro-Wilk W test.

Statistical analyses were performed using MS Office Excel 365, and statistical significance was accepted for $P < 0.05$. Results were expressed as mean ± SD.

Differences in GSI and HSI were assessed by a two tailed Student's t-test for the following groups: wild *vs* captive-reared individuals of the same sex sampled during the same phase of the reproductive cycle. Differences in MMC_d were assessed by a two tailed Student's t-test between the following pair of groups: individuals of the same sex and condition (wild or captive-reared) sampled in the two different phases of the reproductive cycle; wild *vs* captive-reared individuals of the same sex sampled during the same phase of the reproductive cycle. Differences in the MMC_{sz} and MMC_n were analysed by a two tailed Student's t-test between the following groups of females: individuals of the same condition (wild or captive-reared) sampled in the two different phases of the reproductive cycle; wild *vs* captive-reared individuals sampled during the same phase of the reproductive cycle. Differences in AC_d between wild and captive-reared fish sampled during the spawning period were compared by Student's t-test.

The percentage ratio of atretic vitellogenic follicles was compared by χ^2 test in wild *vs* captive-reared fish sampled during the same reproductive phase. The predictive correlation of AF_f with MMC_d as well as the predictive correlations of AC_d with both MMC_d and AF_f were analyzed.

3 RESULTS

Both male and female captive-reared greater amberjack showed significantly lower GSI than wild fish during the spawning phase of the reproductive cycle (Student's t test; $P < 0.05$). No differences in HSI were observed between the two groups (Tables 1 and 2).

The greater amberjack liver (Figure 2a, b) showed polygonal hepatocytes with a moderately basophilic cytoplasm containing lipid droplets of variable size and a spherical euchromatic nucleus with a large central nucleolus, arranged as branched and anastomosed double-cell cords, surrounded by a network of sinusoids. The presence of scattered aggregates of cells and pigments, constituting MMCs (Figure 2b) were visible in most of the examined liver sections. These aggregates contained lipofuscin–ceroids and ferric ions, as demonstrated by Mallory's fuchsin and Perls-Van Gieson stainings (Figure 2c, d).

A diffuse background staining and no immunostaining were observed in liver sections when anti-CYP1A antibodies were diluted 1:50 and 1:500, respectively. A weak anti-CYP1A hepatocyte immunoreactivity was observed in the liver of all the examined specimens using the antibody dilution 1:100. (Figure 3).

Scattered apoptotic cells (Figure 4) were observed in the liver sections of all the examined greater amberjack. No difference in AC_d was found between captive-reared and wild males (0.55 ± 0.28 *vs* 0.40 ± 0.07; Student's t test, $P = 0.29$); whereas captive-reared females showed a six-fold higher AC_d compared with wild females (2.07 ± 1.47 *vs* 0.31 ± 0.19; Student's t test, $P < 0.05$).

Quantitative analysis in wild greater amberjack males indicated that MMC_d decreased significantly (Student's t test, $P < 0.05$) from the gametogenesis to the spawning period (Figure 5a). Differences in MMC_d between wild and captive-reared males were not found, either during the gametogenesis

(Student's t test, p = 0.69) or during the spawning period (Student's t test, p = 0.43). In wild greater amberjack females, no difference was found in MMC_d between the two periods of the reproductive cycle (Student's t test, p = 0.99) (Figure 5b). Conversely, this parameter significantly increased from the gametogenesis to the spawning period in captive-reared individuals (Student's t test, $P < 0.05$). The increase of MMC_d occurring in captive-reared females during the spawning period was due to the increase of both MMC_{sz} and MMC_n (Student's t test, $P < 0.05$) (Figure 6a, b).

Atretic vitellogenic follicles were observed in most of the ovaries containing late vitellogenic follicles. Captive-reared females displayed a significantly higher percentage of atretic vitellogenic follicles compare to wild fish in both examined phases of the reproductive cycle (Table 1; Figure 7).

Significant correlations were found between: AF_f and MMC_d ($MMC_d = 243.71 + 78.44\ AF_f$; $r = 0.884$; $P < 0.01$); AC_d and MMC_d ($MMC_d = 1029.60 + 1109\ AC_d$; $r = 0.677$; $P < 0.05$); AF_f and AC_d ($AC_d = 0.28 + 0.75\ AF_f$; $r = 0.898$; $P < 0.01$).

4 DISCUSSION

The fish liver is a key organ that controls many vital functions and plays a prominent role, both in anabolism and catabolism, as well as in the metabolism of xenobiotics, and it is considered a good indicator of the health status of a fish (Bruslé & Anadon, 1996; Ghosh, Ghosh, & Ray, 2001; Desantis, Corriero, Cirillo, Deflorio, Brill, Griffiths, Lopata, de la Serna, Bridges, Kime, & De Metrio, 2005; Cionna, Maradonna, Olivotto, Pizzonia, & Carnevali, 2006; Kirchhoff, Leef, Ellis, Purser, & Nowak, 2011; Corriero et al., 2013; Passantino et al., 2014). The greater amberjack liver showed the classical architecture with parenchyma arrangement corresponding to the tubular form described by Akiyoshi & Inoue (2004), with hepatocytes arranged in a double layer among sinusoids. The liver parenchyma was characterized by the presence of scattered MMCs containing a) lipofuscin/ceroid pigments that are outputs of the same oxidative polymerization of polyunsaturated fatty acids and may accumulate in fish displaying a wide variety of pathological conditions, including nutritional deficiencies, bacterial and viral diseases, and disturbances caused by toxins (Agius, 1979); and b) ferric iron of

haemosiderin that is considered a residue of the catabolism of several compounds (Leknes, 2004; Ribeiro, Procópio, Gomes, Vieria, Russo, Balzuweit, Chiarini-Garcia, Santana Castro, Rizzo, & Dias Corrêa, 2011) and can be indicative of toxic haemolysis (Pacheco & Santos, 2002).

Melanomacrophage centres are involved in the destruction of endogenous and exogenous material (including dead cells and cell debris) and are associated with apoptosis (Gogal et al., 1999). In the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), the association of MMCs with the exposure to different kinds of stress was proposed (Passantino, Altamura, Cianciotta, Patruno, Tafaro, Jirillo, & Passantino, 2002a; Passantino, Tafaro, Altamura, Arena, Passantino, & Jirillo, 2002b; Passantino, Altamura, Cianciotta, Jirillo, Ribaud, Jirillo, & Passantino, 2004). An increased amount of MMCs in the liver (Passantino et al., 2014), as well as hepatocyte apoptosis and tumour necrosis factor expression (Corriero et al., 2013), were observed in juvenile Atlantic bluefin tuna *Thunnus thynnus* (Linnaeus, 1758) reared in captivity in the North Adriatic Sea, and these conditions were possibly associated with the exposure to persistent organic pollutants and heavy metals. Anchovies *Engraulis encrasicolus* (Linnaeus,1758) sampled from areas exposed to pollutants of industrial/agricultural origin showed liver areas occupied by MMCs and numbers of MMCs significantly higher than those sampled in a marine area not subjected to any known source of pollution (Basilone et al., 2018). Both in Atlantic bluefin tuna (Passantino et al., 2014) and in anchovies potentially exposed to environmental pollution (Basilone et al., 2018), the increased density of liver MMCs was associated with an abnormal liver cytochrome P450 monooxygenase 1A (CYP1A) expression, thus, suggesting an exposure to a variety of organic environmental pollutants (Stegeman, 1978; Husøy, Myers, & Goksøyr, 1996; Jeong & Kim, 2002). Thus, fish exposure to environmental pollutants may result in an increase of both CYP1A expression and MMC density. In particular, after exposure to toxic compounds, increased CYP1A expression may occur for detoxification activity. On the other hand, the evidence of an augmented MMC density may result from tissue damage with involvement of macrophages, which, in turn, aggregate within MMCs following ingestion of damaged cells, dead cells and cell debris (Basilone et al., 2018).

340 Contrary to females, in male greater amberjack we found no difference in the liver area occupied
341 by MMCs between wild and captive-reared individuals. This indicates that fish confinement and
342 handling in captivity *per se* did not cause an increase of MMCs. Moreover, captive-reared fish were
343 not exposed to environmental pollutants as suggested by the weak liver anti-CYP1A immunostaining.
344 Therefore, the present study confirms that fish MMCs represent a sexual dimorphic character
345 (Schwindt, Truelove, Schreck, Fournie, Landers, & Kent, 2006) prompting some speculation for the
346 observed differences between sexes. Captive-reared greater amberjack females showed a significant
347 increase in the amount of liver MMCs from the gametogenesis to the spawning period. Moreover, the
348 liver surface occupied by MMCs in reproductively dysfunctional females during the spawning season
349 was much higher than in wild specimens of the same reproductive stage. Since this was due to an
350 increase in both MMC size and number, the results suggest the rapid formation of new aggregates
351 during the reproductive dysfunction event in addition to the previous ones, ultimately leading to an
352 increased MCCs volume.
353 In female greater amberjack, the observed reproductive dysfunction consisted in the incapacity of
354 the majority of oocytes to finalise vitellogenesis and undergo maturation due to reduced sex hormone
355 secretion (Zupa et al., 2017b, Pousis et al., 2018). During vitellogenesis, oocytes of oviparous
356 vertebrates accumulate large amounts of yolk derived from vitellogenin, a phospholipoprotein
357 synthesized in the liver and released from hepatocytes in the bloodstream to be eventually internalized
358 through receptor-mediated endocytosis (Patiño & Sullivan, 2002; Corriero et al., 2004; Pousis,
359 Santamaria, Zupa, De Giorgi, Mylonas, Bridges, de la Gándara, Vassallo-Agius, Bello, & Corriero,
360 2012; Hara, Hiramatsu, & Fujita, 2016; Pousis et al., 2019). Once internalized in developing oocytes,
361 vitellogenin undergoes proteolytic cleavage to give rise to lipovitellin, phosvitin and β'-component
362 that become part of yolk granules or platelets (Patiño & Sullivan, 2002; Corriero et al., 2004; Hara et
363 al., 2016). In pelagophil species during oocyte maturation, vitellogenin-derived yolk proteins are
364 further hydrolysed to free amino acids, leading to an osmotic flow of water from the maternal plasma
365 to the oocyte (oocyte hydration) (Finn, 2007; Williams, Reading, Hiramatsu, Amano, Glassbrook,

366 Hara, & Sullivan, 2014). Failure of post-vitellogenic oocytes to undergo maturation, either as a
367 physiological event or as a consequence of the pathological impairment of the reproductive function,
368 results in follicular atresia that involves phagocytosis of oocyte components by hypertrophic
369 granulosa cells that act as -and become morphologically similar to- macrophages (Linares-Casenave,
370 Van Eenennaam, & Doroshov, 2002; Corriero et al., 2004; Santos et al., 2008). It has been proposed
371 that yolk-derived proteins are hydrolysed *in situ* to free amino acids (Wood & van der Kraak, 2003);
372 however, there is also clear evidence of a massive transfer of yolk proteins in the bloodstream in the
373 course of follicular atresia (Babin, 1987a, b). The presence of egg yolk proteins in the plasma
374 probably results in their rapid catabolism in organs other than the ovary, such as liver and kidney,
375 which are the two main organs involved in the degradation of high-density lipoproteins in rats
376 (Pittman & Steinherg, 1984). The two mechanisms assure that the energy originally invested in
377 gamete production is not lost. A role of immune cells, granulocytes and macrophages in the resorption
378 of oocytes components during atresia has also been suggested (Besseau & Faliex, 1994).
379 Since the observed increase in liver MMCs and apoptosis did not occur in males (although males
380 did undergo the same handling and captivity-related stress) and fish exposure to toxicants was
381 excluded, here we hypothesize that the hepatic overload related to the metabolism of large amounts
382 of yolk-derived moieties which have been reabsorbed during the process of oocyte atresia may
383 explain our findings. The present study suggests that MMCs may play a role in the metabolism of
384 yolk-derived material reabsorbed during extensive and abnormal follicular atresia in fish, and that
385 changes in MMCs number and size may occur in fish liver as a consequence of massive yolk
386 resorption. Since follicular atresia is a frequent physiological and pathological event in iteroparous
387 teleosts, the present findings suggest that the reproductive state should be always assessed when
388 MMCs are used as markers of exposure to stress or pollutants in wild or farmed fish populations.
389
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CONFLICT OF INTEREST

The authors declare no competing or financial interests.

DATA AVAILABILITY STATEMENT

All the data that support the findings of the present study are reported in Supplementary File 1.

REFERENCES

Agius, C. (1979). The role of melano-macrophage centres in iron storage in normal and disease fish. *Journal of Fish Diseases*, 2, 337-343. https://doi.org/10.1111/j.1365-2761.1979.tb00175.x

Agius, C., & Roberts, R. J. (1981). Effect of starvation on the melanomacrophage centres of fish. *Journal of Fish Biology*, 19, 161-169. https://doi.org/10.1111/j.1095-8649.1981.tb05820.x

Agius, C., & Roberts, R. J. (2003). Melano-macrophage centres and their role in fish pathology. *Journal of Fish Diseases*, 26, 499-509. https://doi.org/10.1046/j.1365-2761.2003.00485.x

Agulleiro, M. J., André, M., Morais, S., Cerdà, J., & Babin, P. J. (2007). High transcript level of fatty acid-binding protein 11 but not of very low-density lipoprotein receptor is correlated to ovarian follicle atresia in a teleost fish (*Solea senegalensis*). *Biology of Reproduction*, 77, 504–516. https://doi.org/10.1095/biolreprod.107.061598

Akiyoshi, H., & Inoue, A. (2004). Comparative histological study of teleost livers in relation to phylogeny. *Zoological Science*, 21, 841–850. https://doi.org/10.2108/zsj.21.841

Babin, P. J. (1987a). Apolipoproteins and the association of egg yolk proteins with plasma high density lipoproteins after ovulation and follicular atresia in the rainbow trout (*Salmo gairdneri*). *The Journal of Biological Chemistry*, 262, 4290-4296.

Babin, P. J. (1987b). Plasma lipoprotein and apolipoprotein distribution as a function of density in the rainbow trout (*Salmo gairdneri*). *Biochemical Journal*, 246, 425-429. https://doi.org/10.1042/bj2460425

Barni, S., Vaccarone, R., Bertone, V., Fraschini, A., Bernini, F., & Fenoglio, C. (2002). Mechanisms of changes to the liver pigmentary component during the annual cycle (activity and hibernation) of *Rana esculenta* L. *Journal of Anatomy*, 200, 185-194. https://doi.org/10.1046/j.0021-8782.2001.00011.x

Basilone, G., Gargano, A., Corriero, A., Zupa, R., Santamaria, N., Mangano, S., Ferreri, R., Pulizzi, M., Mazzola, S., Bonanno, A., & Passantino, L. (2018). Liver melanomacrophage centres and CYP1A expression as response biomarkers to environmental pollution in European anchovy (*Engraulis encrasicolus*) from the western Mediterranean Sea. *Marine Pollution Bulletin*, 131, 197–204. https://doi.org/10.1016/j.marpolbul.2018.04.028

Bauchot, M. L. (1987). Poisson osseux, Familie Carangidae. In W. Fischer, M. L. Bauchot, & M. Schneider (Eds), *Fiches FAO d'identification des espèces pour les besoins de la pêche (Révision 1). Méditerranée et mar Noire. Zone de pêche 37. Volume II. Vertébrés* (1026 pp.). Rome, Food and Agriculture Organization of the United Nations – FAO.

Besseau, L. & Faliex, E. (1994). Resorption of unemitted gametes in *Lithognathus mormyrus* (Sparidae, Teleostei): a possible synergic action of somatic and immune cells. *Cell and Tissue Research*, 276, 123–132. https://doi.org/10.1007/BF00354791

Bruslé, J., & Anadon, G. G. (1996). The structure and function of fish liver. In J. S. D. Munshi & H.M. Dutta (Eds.), *Fish Morfology* (pp.77-93). New York: Science Publisher.

Cervigón, F. (1993). Los peces marinos de Venezuela. Volume 2. Fundación Científica Los Roques, Caracas, Venezuela. 497 p.

Christiansen, J. L., Grzybowski, J. M., & Kodama, R. M. (1996). Melanomacrophage aggregations and their age relationships in the yellow mud turtle, *Kinosternon flavescens* (Kinosternidae). *Pigment Cell Research*, 9, 185-190. https://doi.org/10.1111/j.1600-0749.1996.tb00108.x

Cionna, C., Maradonna, F., Olivotto, I., Pizzonia, G., & Carnevali, O. (2006). Effects of nonylphenol on juveniles and adults in the grey mullet, *Liza aurata*. *Reproductive Toxicology*, 22, 449-454. https://doi.org/10.1016/j.reprotox.2006.04.025

Corriero, A., Acone, F., Desantis, S., Zubani, D., Deflorio, M., Ventriglia, G., Bridges, C. R., Labate, M., Palmieri, G., McAllister, B. G., Kime, D. E., & De Metrio, G. (2004). Histological and immunohistochemical investigation on ovarian development and plasma estradiol levels in the swordfish (*Xiphias gladius* L.). *European Journal of Histochemistry*, 48, 413-421. https://doi.org/10.4081/915

Corriero, A., Desantis, S., Bridges, C. R., Kime, D. E., Megalofonou, P., Santamaria, N., Cirillo, F., Ventriglia, G., Di Summa, A., Deflorio, M., Campobasso, F., & De Metrio, G. (2007). Germ cell proliferation and apoptosis during different phases of swordfish (*Xiphias gladius* L.) spermatogenetic cycle. *Journal of Fish Biology*, 70, 83-99. https://doi.org/10.1111/j.1095-8649.2006.01257.x

Corriero, A., Zupa, R., Bello, G., Mylonas, C. C., Deflorio, M., Genovese, S., Basilone, G., Buscaino, G., Buffa, G., Pousis, C., De Metrio, G., & Santamaria, N. (2011). Evidence that severe acute stress and starvation induce rapid atresia of vitellogenic follicles in Atlantic bluefin tuna, *Thunnus thynnus* (L.) (Osteichthyes: Scombridae). *Journal of Fish Diseases*, 34, 853-860. https://doi.org/10.1111/j.1365-2761.2011.01303.x

Corriero, A., Zupa, R., Pousis, C., Santamaria, N., Bello, G., Jirillo, E., Carrassi, M., De Giorgi, C., & Passantino, L. (2013). Increased liver apoptosis and tumor necrosis factor expression in Atlantic Bluefin tuna (*Thunnus thynnus*) reared in the northern Adriatic Sea. *Marine Pollution Bulletin*, 71, 23-28. https://doi.org/10.1016/j.marpolbul.2013.03.041

Desantis, S., Corriero, A., Cirillo, F., Deflorio, M., Brill, R., Griffiths, M., Lopata, A. L., de la Serna, J. M., Bridges, C. R., Kime, D. E., & De Metrio, G. (2005). Immunohistochemical localization of CYP1A, vitellogenin and Zona radiata proteins in the liver of swordfish (*Xiphias gladius* L.) taken from the Mediterranean Sea, South Atlantic, South Western Indian and Central North Pacific Oceans. *Aquatic Toxicology*, 71, 1-12. https://doi.org/10.1016/j.aquatox.2004.10.005

Evans, D., & Nowak, B. (2016). Effect of ranching time on melanomacrophage centres in anterior kidney and spleen of Southern bluefin tuna, *Thunnus maccoyii*. *Fish & Shellfish Immunology*, 59, 358-364. http://dx.doi.org/10.1016/j.fsi.2016.11.014

Fakriadis, I., Lisi, F., Sigelaki, I., Papadaki, M., & Mylonas, C. C. (2019). Spawning kinetics and egg/larval quality of greater amberjack (*Seriola dumerili*) in response to multiple GnRHa injections or implants. *General and Comparative Endocrinology*, 279, 78-87. https://doi.org/10.1016/j.ygcen.2018.12.007

Finn, R. N. (2007). The maturational disassembly and differential proteolysis of paralogous vitellogenins in a marine pelagophil teleost: A conserved mechanism of oocyte hydration. *Biology of Reproduction*, 76, 936–948. https://doi.org/10.1095/biolreprod.106.055772

Fishelson, L. (2006). Cytomorphological alterations of the thymus, spleen, head-kidney, and liver in cardinal fish (Apogonidae, Teleostei) as bioindicators of stress. *Journal of Morphology*, 267, 57-69. https://doi.org/10.1002/jmor.10385

Fournie, J. W., Summers, J. K., Courtney, L. A., & Engle, W. D. (2001). Utility of splenic macrophage aggregates as an indicator of fish exposure to degraded environments. *Journal of Aquatic Animal Health*, 13, 105-116. https://doi.org/10.1577/1548-8667(2001)013<0105:UOSMAA>2.0.CO;2

Ghosh, M. C., Ghosh, R., & Ray, A. K. (2001). Impact of copper on biomonitoring enzyme ethoxyresorufin-o-deethylase in cultured catfish hepatocytes. *Environmental Research*, 86, 167-173. https://doi.org/10.1006/enrs.2001.4249

Gogal, R. M. Jr., Smith, B. J., Robertson, J. L., Smith, S. A., & Holladay, S. D. (1999). Tilapia (*Oreochromis niloticus*) dosed with azathioprine display immune effects similar to those seen in mammals, including apoptosis. *Veterinary Immunology and Immunopathology*, 68, 209-227. https://doi.org/10.1016/S0165-2427(99)00024-0

Guraya, S. S. (1986). The cell and molecular biology of fish oogenesis. *Monographs in Developmental Biology Vol. 18*. Basel, CH: Karger.

Hara, A., Hiramatsu, N., & Fujita, T. (2016). Vitellogenesis and choriogenesis in fishes. *Fisheries Science*, 82, 187–202. https://doi.org/10.1007/s12562-015-0957-5

Hunter, J. R., & Macewicz, B. J. (1985). Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax*. *Fishery Bulletin*, 83, 119 136.

Husøy, A.-M., Myers, M., & Goksøyr, A. (1996). Cellular localization of cytochrome P450 (CYP1A) induction and histology in Atlantic cod (*Gadus morhua* L.) and European flounder (*Platichthys flesus*) after environmental exposure to contaminants by caging in Sørfjorden, Norway. *Aquatic Toxicology*, 36, 53-74. https://doi.org/10.1016/S0166-445X(96)00797-7

Jeong, H. G., & Kim, J. Y. (2002). Effects of *o,p'*-DDT on the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible CYP1A1 expression in murine Hepa-1c1c7 cells. *Food and Chemical Toxicology*, 40, 1685-1692. https://doi.org/10.1016/S0278-6915(02)00099-6

517 Jørgensen, C., Ernande, B., Fiksen, Ø., & Dieckmann, U. (2006). The logic of skipped spawning in
518 fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 63, 200–211.
519 https://doi.org/10.1139/f05-210
520 Kirchhoff, N. T., Leef, M. J., Ellis, D., Purser, J., & Nowak, B. F. (2011). Effects of the first two
521 months of ranching on the health of southern bluefin tuna *Thunnus maccoyii*. *Aquaculture*, 315,
522 207-212. https://doi.org/10.1016/j.aquaculture.2011.02.036
523 Koppang, E. O., Haugarvoll, E., Hordvik, I., Aune, L., & Poppe, T. T. (2005). Vaccine-associated
524 granulomatous inflammation and melanin accumulation in Atlantic salmon, *Salmo salar* L., white
525 muscle. *Journal of Fish Diseases*, 28, 13-22. https://doi.org/10.1111/j.1365-2761.2004.00583.x
526 Krysko, D. V. Diez-Fraile, A., Criel, G., Svistunov, A. A., Vandenabeele, P., & D'Herde, K. (2008).
527 Life and death of female gametes during oogenesis and folliculogenesis. *Apoptosis*, 13, 1065-
528 1087. https://doi.org/10.1007/s10495-008-0238-1
529 Leknes, I. L. (2004). Melano-macrophage centres in the liver of platyfish, *Xiphophorus maculatus*,
530 Poeciliidae: Teleostei. *Zoology*, 107, 201-204. https://doi.org/10.1016/j.zool.2004.07.002
531 Linares-Casenave, J., Van Eenennaam, J. P., & Doroshov, S. I. (2002). Ultrastructural and
532 histological observations on temperature-induced follicular ovarian atresia in the white sturgeon.
533 *Journal of Applied Ichthyology*, 18, 382–390. https://doi.org/10.1046/j.1439-0426.2002.00369.x
534 Loumbourdis, N. S., & Vogiatzis, A. K. (2002). Impact of cadmium on liver pigmentary system of
535 the frog *Rana ridibunda*. *Ecotoxicology and Environmental Safety*, 53, 52-58.
536 https://doi.org/10.1006/eesa.2002.2153
537 Mazzi, V. (1977). Manuale di tecniche istologiche e istochimiche. Piccin (Ed), Padova, Italy. 750 p.
538 Mela, M., Randi, M. A., Ventura, D. F., Carvalho, C. E., Pelletier, E., & Oliveira Ribeiro, C. A.
539 (2007). Effects of dietary methylmercury on liver and kidney histology in the neotropical fish
540 *Hoplias malabaricus*. *Ecotoxicology and Environmental Safety*, 68, 426-435.
541 https://doi.org/10.1016/j.ecoenv.2006.11.013
542 Metcalfe, J. D., & Craig, J. F. (2011). Ethical justification for the use and treatment of fishes in
543 research: an update. *Journal of Fish. Biology*, 78, 393-394. https://doi.org/10.1111/j.1095-
544 8649.2010.02900.x
545 Miranda, A. C. L., Bazzoli, N., Rizzo, E., & Sato, Y. (1999). Ovarian follicular atresia in two teleost
546 species: a histological and ultrastructural study. *Tissue and Cell*, 31, 480-488.
547 https://doi.org/10.1054/tice.1999.0045
548 Mylonas, C. C., Cardinaletti, G., Sigelaki, I., & Polzonetti-Magni, A. (2005). Comparative efficacy
549 of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass

(*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. *Aquaculture*. 246, 467-481. https://doi.org/10.1016/j.aquaculture.2005.02.046

Pacheco, M., & Santos, M. A. (2002). Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). *Ecotoxicology and Environmental Safety*, 53, 331–347. https://doi.org/10.1016/S0147-6513(02)00017-9

Passantino, L., Altamura, M., Cianciotta, A., Jirillo, F., Ribaud, M. R., Jirillo, E., & Passantino, G. F. (2004). Maturation of fish erythrocytes coincides with changes in their morphology, enhanced ability to interact with *Candida albicans* and release of cytokine-like factors active upon autologous macrophages. *Immunopharmacology and Immunotoxicology*, 26, 573-585. https://doi.org/10.1081/IPH-200042323

Passantino, L., Altamura, M., Cianciotta, A., Patruno, R., Tafaro, A., Jirillo, E., & Passantino, G. F. (2002a) Fish Immunology I. Binding and engulfment of *Candida albicans* by erythrocytes of rainbow trout (*Salmo gairdneri* Richardson). *Immunopharmacology and Immunotoxicology*, 24, 665-678. https://doi.org/10.1081/IPH-120016050

Passantino, L., Santamaria, N., Zupa, R., Pousis, C., Garofalo, R., Cianciotta, A., Jirillo, E., Acone, F., & Corriero, A. (2014). Liver melanomacrophage centres as indicators of Atlantic bluefin tuna, *Thunnus thynnus* L. well-being. *Journal of Fish Diseases*, 37, 241-250. https://doi.org/10.1111/jfd.12102

Passantino, L., Tafaro, A., Altamura, M., Arena, R., Passantino, G. F., & Jirillo, E. (2002b). Fish Immunology II. Morphological and cytochemical characterization and phagocytic activities of head kidney macrophages from rainbow trout (*Salmo gairdneri* Richardson). *Immunopharmacology and Immunotoxicology*, 24, 679-691.

Patiño, R., & Sullivan, C. V. (2002). Ovarian follicle growth, maturation, and ovulation in teleost fish. *Fish Physiology and Biochemistry*, 26, 57–70. https://doi.org/10.1023/A:1023311613987

Paxton, J. R., Hoese, D. F., Allen, G. R., & Hanley, J. E. (1989). Pisces. Petromyzontidae to Carangidae. *Zoological Catalogue of Australia, Vol. 7*. Canberra, AU: Australian Government Publishing Service.

Phromkunthong, W., Nuntapong, N., Wanlem, S., & Boonyaratpalin, M. (2015). A study on growth, histopathology and oxidative stress in Asian sea bass on diets with various loadings of melamine and cyanuric acid adulterants, *Aquaculture*, 435, 336-346. https://doi.org/10.1016/j.aquaculture.2014.10.009

Pittman, R. C., & Steinherg, D. (1984). Sites and mechanisms of uptake and degradation of high density and low density lipoproteins. *Journal of Lipid Research*, 25, 1577-1585.

583 Pousis, C., Mylonas, C. C., De Virgilio, C., Gadaleta, G., Santamaria, N., Passantino, L., Zupa, R.,
584 Papadaki, M., Fakriadis, I., Ferreri, R., & Corriero, A. (2018). The observed oogenesis
585 impairment in greater amberjack *Seriola dumerili* (Risso, 1810) reared in captivity is not related
586 to an insufficient liver transcription or oocyte uptake of vitellogenin. *Aquaculture Research*, 49,
587 243-252. https://doi.org/10.1111/are.13453
588 Pousis, C., Rodríguez, C., De Ruvo, P., De Virgilio, C., Pérez, J., Mylonas, C. C., Zupa, R.,
589 Passantino, L., Santamaria, N., Valentini, L., & Corriero, A. (2019). Vitellogenin receptor and
590 fatty acid profiles of individual lipid 1 classes of oocytes from wild and captive-reared greater
591 amberjack (*Seriola dumerili*) during the reproductive cycle. *Theriogenology*, 140, 73-83.
592 https://doi.org/10.1016/j.theriogenology.2019.08.014
593 Pousis, C., Santamaria, N., Zupa, R., De Giorgi, C., Mylonas, C. C., Bridges, C. R., de la Gándara,
594 F., Vassallo-Agius, R., Bello, G., & Corriero, A. (2012). Expression of vitellogenin receptor gene
595 in the ovary of wild and captive Atlantic bluefin tuna (*Thunnus thynnus*). *Animal Reproduction*
596 *Science*, 132, 101-110. https://doi.org/10.1016/j.anireprosci.2012.03.014
597 Ribeiro, H. J., Procópio, M. S., Gomes, J. M. M., Vieria, F. O., Russo, R. C., Balzuweit, K., Chiarini-
598 Garcia, H., Santana Castro, A. C., Rizzo, E., & Dias Corrêa, J. Jr. (2011). Functional dissimilarity
599 of melanomacrophage centres in the liver and spleen from females of the teleost fish *Prochilodus*
600 *argenteus*. *Cell and Tissue Research*, 346, 417-425. https://doi.org/10.1007/s00441-011-1286-3
601 Rizzo, E., & Bazzoli, N. (1995). Follicular atresia in curimatá-pioa *Prochilodus affinis* Reinhardt,
602 1874 (Pisces, Characiformes). *Revista Brasileira de Biologia*, 55, 697-703.
603 Roberts, R. J. (1975). Melanin containing cells of teleost fish and their relation to disease. In W. E.
604 Ribelin & G. Migaki (Eds.), *Fish Pathology* (pp. 399-428). Madison: University of Wisconsin
605 Press.
606 Rund, C. R., Christiansen, J. L., & Johnson, J. C. (1998). In vitro culture of melanomacrophages from
607 the spleen and liver of turtles: comments on melanomacrophage morphology. *Pigment Cell*
608 *Research*, 11, 114-119. https://doi.org/10.1111/j.1600-0749.1998.tb00720.x
609 Santos, H. B., Thomé, R. G., Arantes, F. P., Sato, Y., Bazzoli, N., & Rizzo, E. (2008). Ovarian
610 follicular atresia is mediated by heterophagy, autophagy, and apoptosis in *Prochilodus argenteus*
611 and *Leporinus taeniatus* (Teleostei: Characiformes). *Theriogenology*, 70, 1449–1460.
612 https://doi.org/10.1016/j.theriogenology.2008.06.091
613 Schwindt, A. R., Truelove, N., Schreck, C. B., Fournie, J. W., Landers, D. H., & Kent, M. L. (2006).
614 Quantitative evaluation of macrophage aggregates in brook trout *Salvelinus fontinalis* and
615 rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms*, 68, 101–113.
616 https://doi.org/10.3354/dao068101

Smith, C. L. (1997). *National Audubon Society field guide to tropical marine fishes of the Caribbean, the Gulf of Mexico, Florida, the Bahamas, and Bermuda*. New York, NY: Alfred A. Knopf, Inc.

Smith-Vaniz, W. F., Pina Amargos, F., Brown, J., Curtis, M., & Williams, J. T. (2015). *Seriola dumerili* (errata version published in 2017). The IUCN Red List of Threatened Species 2015: e.T198643A115341394. http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T198643A16644002.en. Downloaded on 30 September 2019.

Stegeman, J. J. (1978). Influence of environmental contamination on cytochrome P-450 mixed function oxygenase in fish; implication for recovery in the Wild Harbour marsh. *Journal of Fisheries Research Board of Canada*, 35, 668-674. https://doi.org/10.1139/f78-117

van der Oost, R., Beyer, J., & Vermeulen, N. P. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology*, 13, 57-149. https://doi.org/10.1016/S1382-6689(02)00126-6

van der Weiden, M. E. J., Bleumink, R., Seinen, W., & van den Berg, M. (1994). Concurrence of P450 1A induction and toxic effects in the mirror carp (*Cyprinus carpio*), after administration of allow dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Aquatic Toxicology*, 29, 147-162. https://doi.org/10.1016/0166-445X(94)90065-5

Williams, V. N., Reading, B. J., Hiramatsu, N., Amano, H., Glassbrook, N., Hara, A., & Sullivan, C. V. (2014). Multiple vitellogenins and product yolk proteins in striped bass, *Morone saxatilis*: molecular characterization and processing during oocyte growth and maturation. *Fish Physiology and Biochemistry*, 40, 395–415. https://doi.org/10.1007/s10695-013-9852-0

Wolke, R. E. (1992). Piscine macrophage aggregates, a review. *Annual Review of Fish Diseases*, 2, 91–108. https://doi.org/10.1016/0959-8030(92)90058-6

Wood, A.W., & Van Der Kraak, G. (2003). Yolk proteolysis in rainbow trout oocytes after serum-free culture: evidence for a novel biochemical mechanism of atresia in oviparous vertebrates. *Molecular Reproduction and Development*, 65, 219–227. https://doi.org/10.1002/mrd.10272

Yunis-Aguinaga, J., Claudiani, G. S., Marcusso, P. F., Manrique, W. G., Engrácia de Moraes, J. R., de Moraes, F. R., & Fernandes, J. B. K. (2015). *Uncaria tomentosa* increases growth and immune activity in *Oreochromis niloticus* challenged with *Streptococcus agalactiae*. *Fish & Shellfish Immunology*, 47, 630-638. https://doi.org/10.1016/j.fsi.2015.09.051

Zhang, X., Li, Y.-W., Mo, Z.-Q., Luo, X.-C., Sun, H.-Y., Liu, P., Li, A.-X., Zhou, S.-M., & Dan, X.-M. (2014). Outbreak of a novel disease associated with *Vibrio mimicus* infection in freshwater cultured yellow catfish, *Pelteobagrus fulvidraco*. *Aquaculture*, 432, 119-124. https://doi.org/10.1016/j.aquaculture.2014.04.039

Zupa, R., Fauvel, C., Mylonas, C. C., Pousis, C., Santamaria, N., Papadaki, M., Fakriadis, I., Cicirelli, V., Mangano, S., Passantino, L., Lacalandra, G. M., & Corriero, A. (2017a). Rearing in captivity affects spermatogenesis and sperm quality in greater amberjack *Seriola dumerili* (Risso, 1810). *Journal of Animal Science*, 95, 4085-4100. https://doi.org/10.2527/jas.2017.1708

Zupa, R., Rodríguez, C., Mylonas, C. C., Rosenfeld, H., Fakriadis, I., Papadaki, M., Pérez, J. A., Pousis, C., Basilone, G., & Corriero, A. (2017b). Comparative study of reproductive development in wild and captive-reared greater amberjack *Seriola dumerili* (Risso,1810). *PLoS ONE* 12(1): e0169645. https://doi.org/10.1371/journal.pone.0169645

Figure legends

FIGURE 1 (a) Ventral view of greater amberjack viscera. Asterisk, spleen; H, hearth; L, liver; PC, pyloric ceca; S, stomach. (b) Dorsal view of greater amberjack liver. The arrow points to the hilum; the circle indicates the area from which liver samples were taken.

FIGURE 2 Micrographs of greater amberjack liver sections. (a) General structure of the liver showing hepatocytes containing lipid droplets of variable size, blood vessels and sinusoids. Mallory's trichrome staining. (b) Melanomacrophage centre in close proximity to a vein. Mallory's trichrome staining. c) Melanomacrophage centre stained with Mallory's fuchsin method, showing a surrounding connective capsule and some monocyte-macrophages. (d) Melanomacrophage centre stained with Perls-Van Gieson staining for ferric iron of hemosiderin. Scale bars = 100 µm in (a), 50 µm in (b) and (d), 20 µm in (c). Arrowhead, monocyte-macrophage; c, connective capsule; mmc, melanomacrophage centre, s, sinusoid.

FIGURE 3 Micrographs of liver sections immunostained with anti-cytochrome P450 monooxygenase 1A (CYP1A) antibodies. (a) No immunostaining was observed in greater amberjack liver sections when the primary antibody was omitted (negative control). (b) Weak immunoreaction observed in hepatocytes of both wild and captive-reared greater amberjack. (c) Intense immunoreaction in hepatocytes from European anchovy *Engraulis encrasicolus* sampled in an area exposed to pollutants of industrial/agricultural origin (positive control) (Basilone et al., 2018). Scale bars = 50 µm.

FIGURE 4 Apoptotic hepatocytes (arrowheads) in a liver section stained with the terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate nick end labeling (TUNEL). Scale bars = 50 µm.

FIGURE 5 Mean (± SD) liver surface occupied by melanomacrophages centres (MMC_d) in wild and captive-reared greater amberjack. Asterisks indicate statistically significant differences versus the preceding phase of the reproductive cycle. Different letters indicate significant differences between wild and captive-reared females in the same phase of the reproductive cycle (Student's t-test; $P < 0.05$).

692 **FIGURE 6** Mean (± SD) size (MMC_{sz}) (a) and number (MMC_n) (b) of melanomacrophages centres
693 in wild and captive-reared greater amberjack females. Asterisk indicates statistically significant
694 differences versus the preceding reproductive phase. Different letters indicate significant differences
695 between wild and captive-reared females in the same phase of the reproductive cycle (Student's t-
696 test; $P < 0.05$).
697
698 **FIGURE 7** Micrographs of greater amberjack ovary sections stained with haematoxylin-eosin. (a)
699 Ovary section from a wild fish sampled during the gametogenesis phase; no atretic vitellogenic
700 follicles are visible. (b) Ovary section from a captive-reared fish sampled during the gametogenesis
701 phase displaying about 50% of atretic vitellogenesis follicles. (c) Ovary section of a captive-reared
702 females sampled during the spawning phase of the reproductive cycle showing extensive (100%)
703 atresia of vitellogenesis follicles. Scale bars = 400 µm. af, atretic vitellogenic follicles; lv, late
704 vitellogenic oocytes.
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