New species for EU aquaculture

Deliverable Report

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Objective

Description of the process of oogenesis in captive-reared vs hatchery-produced grey mullet, including aspects of growth, body indices, and histological evaluation of ovarian development: account of captive effects on pubertal development in grey mullet populations. With the aim of highlighting potential correlations between domestication and improved growth and maturational processes, the deliverable will include a comparative documentation of first sexual maturity and growth performance in wild and hatchery-produced grey mullet specimens reared in captivity.

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Description

Puberty is the developmental period comprising the transition from an immature juvenile to a mature adult state of the reproductive system, i.e. the stage of development during which an individual becomes capable of reproducing sexually (Taranger et al., 2010). As in other vertebrates, puberty in fish is affected by the interaction between environmental and genetic factors, and necessitates the full activation of the brain–pituitary–gonadal (BPG) axis, which depends largely on the coordinated functions of the two pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Carrillo et al., 2009; Taranger et al., 2010; Berkovich et al., 2013).

For fish farming industry, both early and delayed puberty may represent a major problem. The occurrence of a precocious puberty affects growth, health and welfare in salmonids (McClure et al., 2007), sea basses (Felip et al., 2008), flatfishes (Weltzien et al., 2003), cod fishes (Karlsen et al., 2006), tilapias (Longalong et al., 1999), sea breams (Gines et al., 2003, 2004) and perches (Shewmon et al., 2007). On the contrary, a delay or failure in the attainment of puberty may prevent reproduction and closure of the life-cycle in culture (Dufour et al., 2003; van Ginneken et al., 2007). In some cases (i.e. sturgeons), an advanced puberty increases farming economic sustainability by accelerating the production cycle of high-value products such as caviar (Taranger et al., 2010). Within a few years, the development of a technology to induce a precocious puberty could significantly increase grey mullet culture profitability, because it would allow to produce roe (bottarga, i.e. salted, cured ovaries), a highly appreciated niche delicacy that, in some countries, reaches market quotations around 100 €/kg and is now obtained only from wild fish.

The present deliverable provides a description of the reproductive cycle of female grey mullet in the Mediterranean and compare the first sexual maturity and growth performance in wild and hatchery-produced grey mullet specimens reared in captivity.

Background

The Mugilidae, commonly known as grey mullets, are an ubiquitous teleost family occurring in most temperate, sub-tropical and tropical coastal waters in both hemispheres (Crosetti and Blabber, 2016). There is increasing evidence that *Mugil cephalus* is part of a species complex (Shen et al., 2011) including at least 14 Mugil species (Durand et al., 2012). These species occupy a wide variety of marine, estuarine and freshwater environments but spawning occurs in the sea (Thomson, 1955; Ibáñez and Gutiérrez-Benítez, 2004). They are gonochoristic or bisexual fish (González-Castro and Minos, 2016), although they can sometime exhibit non-functional hermaphroditism (McDonough et al., 2005).

Grey mullets show a group-synchronous ovarian development (Bartulović et al., 2011) and spawning in the Mediterranean occurs in late summer-early autumn (Assem et al., 2008; Bartulović et al., 2011). The size at sexual maturity ranges very widely, with males usually maturing between 25 and 30 cm standard length (SL) and females slightly larger at 27–35 cm SL (Ameur et al., 2003). These size classes are generally regarded as being approximately 3 years old but some studies have given higher and lower ages at 50 % sexual maturity (Bok, 1983; Ameur et al., 2003). The quite wide range in the above estimates can be possibly ascribed to both differences in the methodology used by the different authors and to the fact that they actually referred to different taxa within the *M. cephalus* species complex (Withfield et al., 2012.).

The culture of grey mullet is mostly dependent on the availability of wild fry as breeding in captive condition is not standardized (Kumar et al., 2015). Among the major bottlenecks for the incorporation of a new species in the aquaculture industry, reproductive dysfunctions affect frequently fish in captivity, hindering the production of viable eggs. Reproductive dysfunctions commonly involve an inadequate pituitary GtHs synthesis and/or release (Zohar and Mylonas, 2001; Mylonas et al., 2010; Berkovich et al., 2013), which has been attributed to captivity-induced stress, lack of suitable environmental conditions (Mylonas et al., 2010) and/or nutritional deficiencies (Izquierdo et al., 2001).

When reared in captivity, grey mullets display severe reproductive dysfunctions (Yashouv, 1969; De Monbrison et al., 1997): spermiating males are rarely observed and, in most cases, the produced milt is highly

viscous and fails to fertilize the eggs; females are unable to finalize vitellogenesis or fail to undergo oocyte maturation once vitellogenesis is completed (De Monbrison et al., 1997). The observed failure in the attainment of vitellogenesis not only prevents ovulation and spawning but also makes cultured grey mullets unsuitable for the production of bottarga.

Treatments with controlled-release GnRHa implants combined with dopamine antagonist and 17α-methyl-testosterone (Aizen et al 2005) or with recombinant tuna FSH (Rosenfeld et al., 2011) have been proved to be effective in ameliorating the observed reproductive dysfunctions. Within DIVERSIFY, further work aimed at the setup of a suitable hormonal therapy for the grey mullet reproduction control has been carried out by P.4 IOLR and it will be described in D. 7.1.

Materials and Methods

The lists of all the grey mullet specimens used for the present Deliverable are reported in **Table 1** and **Table 2** for wild and farmed specimens, respectively. From the sampled fish, the following data were recorded: total length, TL in cm; body mass, BM in g; gonad mass, GM in g. The gonado-somatic index was calculated as $GSI = 100xGMxBM^{-1}$.

In order to compare body growth in wild and farmed grey mullet, the age of 16 specimens sampled in the Lesina lagoon (10 females and 6 males) was determined through the count of growth marks observed in their scales. To this aim, a variable number of scales were gently removed from the skin taken from a body area between the pectoral and first dorsal fin. The scales were rinsed in tap water and in 70% ethanol and finally placed between two microscope slides (McCurdy et al., 2002). The scales were observed with a binocular lens microscope Wild M3C (Leitz, Heerbrugg, Switzerland) under transmitted light, connected through a digital camera DC 300 (Leica, Wetzlar, Germany) to the image analyser Quantiment 500 W (Leica, Wetzlar, Germany). Scales show typical dense concentric growth rings (circuli) whose arrangement displays periodical (seasonal) variations: circuli density increases and circuli crowd during the slow growth season (winter) when they tend to form a solid line or annulus. The age of the fish was estimated based on the number of annuli counted on its scales (Meunier, 2002).

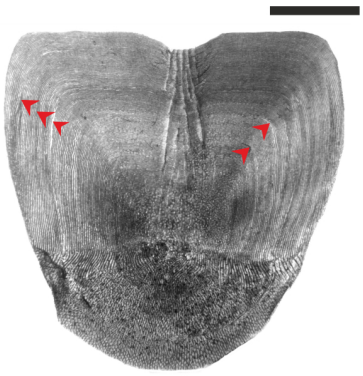

Figure 1. Scale from a 5-year old wild grey mullet. Bar = 3 mm. Arrowheads point to annuli.

Estimate of grey mullet theoretical growth in length was obtained by fitting the von Bertalanffy growth model (Bertalanffy von, 1938) to the mean lengths at estimated age:

$$TL_t = TL_\infty [1 - e^{-k(t-t_0)}]$$

where, TL_t = predicted fork length at age t; TL_∞ = mean asymptotic fork length; k = growth constant ($year^{-1}$); and t_0 = theoretical age at which the fish would have been 0 cm in length.

For the analysis of body condition, 2 years old (hereafter referred to as age 2; n = 21) and 3 years old (hereafter referred to as age 3; n = 19) grey mullets were used. Each of the two age groups included two sub-groups: one of them was constituted by fish caught from the wild in the Ebro delta (northeastern Spanish coast) at an early stage, transferred to IOLR facility (Eilat, Israel) and reared in captivity for 2 or 3 years; the other group was made by fish produced in IOLR hatchery and reared in the same facility for 2 or 3 years before sampling. Moreover, both captive-reared and hatchery-produced fish were reared in 19 m^3 tanks at two different densities (low density =45 fish/m^3; high density = 90 fish /m^3) in order to evaluate the effect of rearing density on ovarian development and maturation.

As indicator of fish body condition, Fulton's condition factor (K) was calculated according the following equation:

$K = BM\ 100/TL^3$

For the description of oogenesis, for the study of the seasonal pattern of ovarian development of grey mullet in the wild as well as of the effect of different rearing conditions on ovarian development, ovary samples were cut and fixed in Bouin's solution, dehydrated in ethanol, clarified in xylene and embedded in paraffin wax. Five-µm thick sections were stained with hematoxylin-eosin and observed under a light microscope. The reproductive state was assessed by recording the most advanced oocyte stage for each specimen, according to commonly used classifications (Corriero et al., 2007; Zupa et al., 2017) (**Tables 1 and 2**).

In order to compare oocyte yolk accumulation in wild, captive-reared and hatchery-produced individuals, the largest vitellogenic oocytes, having a large and centrally located nucleus were selected. Oocyte diameter (µm) and surface occupied by yolk granules (µm^2) were measured from microphotographs taken with a digital camera (DFC 420; Leica, Cambridge, UK) connected to a light microscope (DIAPLAN; Leitz, Wetzlar, Germany), using an image analysis software (Leica Application Suite, version 3.3.0; Cambridge, UK).

All the results are presented as means ± SE; the statistical probability significance was established at the $P < 0.05$ level. Differences of biometric data (TL, BM, GSI) and Fulton's condition factor were assessed between the following pair of groups by a two tailed Student's t-test: wild specimens sampled in two consecutive months; captive-reared *vs* hatchery-produced specimens; fish reared at low *vs* fish reared at high density. Differences in the surface occupied by yolk granules of late vitellogenic oocytes among wild, captive-reared and hatchery-produced grey mullet was assessed by one-way ANOVA, followed by the Tukey-Kramer multiple-comparison test.

Table 1. Biometric data, maturity stage and age of wild grey mullet.

Fish origin	Sampling area	Sampling date	Total Length (TL, cm)	Body Mass (BM, g)	Gonad Mass (GM, g)	Most advanced oocyte stage	Age (years)
Wild	Ionian Sea	18/05/2014	41.2	670	4.2	perinucleolar	n.a.
		18/05/2014	48.5	1100	8.4	perinucleolar	n.a.
		18/05/2014	54.5	1570	14.8	lipid/cortical alveoli	n.a.
		28/05/2014	40.6	640	2.0	perinucleolar	n.a.
		28/05/2014	43.3	780	3.8	perinucleolar	n.a.
		28/05/2014	49.1	1140	6.5	perinucleolar	n.a.
		29/05/2014	46.2	950	5.7	perinucleolar	n.a.
		14/06/2014	41.6	690	2.9	perinucleolar	n.a.
		14/06/2014	42.6	740	3.8	lipid/cortical alveoli	n.a.
		14/06/2014	46.4	960	6.0	lipid/cortical alveoli	n.a.
		25/06/2014	46.9	990	7.0	lipid/cortical alveoli	n.a.
		25/06/2014	52.8	1420	8.7	perinucleolar	n.a.
		25/06/2014	53.9	1520	10.7	lipid/cortical alveoli	n.a.
		25/06/2014	55.1	1620	15.2	lipid	n.a.
		17/07/2014	46.1	940	2.4	perinucleolar	n.a.
		17/07/2014	50.9	1270	7.7	lipid/cortical alveoli	n.a.
		22/07/2014	45.3	890	5.8	lipid/cortical alveoli	n.a.
		22/07/2014	46.2	950	12.4	early vitellogenesis	n.a.
		22/07/2014	48.2	1080	9.5	early vitellogenesis	n.a.
		24/07/2014	39.5	590	4.1	early vitellogenesis	n.a.
		24/07/2014	39.5	590	2.5	perinucleolar	n.a.
	Divari lagoon	19/08/2014	39.1	570	1.5	perinucleolar	n.a.
		19/08/2014	40.4	630	3.3	perinucleolar	n.a.
		19/08/2014	41.8	700	1.5	perinucleolar	n.a.
	Ionian Sea	22/08/2014	47.3	1020	240.0	late vitellogenesis	n.a.
		22/08/2014	47.9	1060	300.0	late vitellogenesis	n.a.
		22/08/2014	48.7	1110	230.0	late vitellogenesis	n.a.
		22/08/2014	49.2	1150	15.1	lipid/cortical alveoli (pathological ?)	n.a.
		29/08/2014	44.9	870	170.0	late vitellogenesis	n.a.
	Divari lagoon	29/08/2014	46.4	960	200.0	late vitellogenesis	n.a.
		29/08/2014	47.6	1040	210.0	late vitellogenesis	n.a.
Wild	Lesina lagoon	09/09/2016	38.0	575	111.3	late vitellogenesis	4
		09/09/2016	40.0	603	96.8	late vitellogenesis	4
		09/09/2016	42.0	865	9.3	early vitellogenesis	5
		09/09/2016	47.0	1072	232.6	late vitellogenesis/atretic follicles	6
		09/09/2016	50.0	1332	280.4	late vitellogenesis	6
		13/09/2016	46.0	843	150.0	late vitellogenesis	5
		13/09/2016	49.0	1132	245.0	late vitellogenesis	6
		14/09/2016	40.0	595	108.0	late vitellogenesis	4
		14/09/2016	43.0	807	166.7	late vitellogenesis	5
		14/09/2016	45.0	798	154.9	late vitellogenesis	5
		13/09/2016*	37.0	477	20.3		4
		14/09/2016*	38.0	473	14.0		4
		14/09/2016*	38.0	528	22.8		4
		09/09/2016*	41.0	633	17.4		5
		09/09/2016*	41.0	640	29.2		5
		13/09/2016*	45.0	942	27.5		5

Asterisk: male specimens used only for age and growth analyses. The total length of the wild specimens sampled in the Divari lagoon and in the Ionian Sea was not measured during sampling and it was estimated from the length-weight relationship obtained from the other wild specimens. n.a. = not available.

Table 2. Biometric data, maturity stage and age of captive-reared and hatchery produced grey mullet sampled at P4. IOLR (Israel).

Fish origin	Rearing density	Sampling date	Total Length (TL, cm)	Body mass (BM, g)	Gonad mass (GM, g)	Most advanced oocyte stage	Age (years)
captive-reared	low	03/11/2016	22.2	100	0.1	perinucleolar	2
		03/11/2016	23.3	120	0.1	perinucleolar	2
		03/11/2016	25.3	150	0.1	perinucleolar	2
		03/11/2016	36.2	520	1.2	perinucleolar	2
		13/09/2017	32.1	330	2.3	perinucleolar	3
		13/09/2017	36.7	650	11.7	late vitellogenesis	3
		13/09/2017	50.2	1330	21.3	perinucleolar	3
captive-reared	high	03/11/2016	23.0	120	0.2	perinucleolar	2
		13/09/2017	37.0	500	1.2	perinucleolar	3
		13/09/2017	41.4	750	2.7	perinucleolar	3
		13/09/2017	43.2	1030	64.1	late vitellogenesis	3
hatchery produced	low	03/11/2016	29.1	240	0.4	perinucleolar	2
		03/11/2016	29.5	270	0.6	perinucleolar	2
		03/11/2016	31.0	270	0.4	perinucleolar	2
		03/11/2016	31.0	270	0.7	perinucleolar	2
		03/11/2016	32.5	310	0.6	perinucleolar	2
		03/11/2016	32.6	340	0.9	perinucleolar	2
		03/11/2016	32.9	340	0.9	perinucleolar	2
		03/11/2016	32.9	330	0.7	perinucleolar	2
		13/09/2017	29.6	240	0.6	perinucleolar	3
		13/09/2017	34.7	390	1.0	perinucleolar	3
		13/09/2017	34.9	470	1.6	perinucleolar	3
		13/09/2017	36.5	580	102.0	late vitellogenesis	3
		13/09/2017	38.5	525	51.4	late vitellogenesis	3
		13/09/2017	43.5	780	122.3	late vitellogenesis	3
		13/09/2017	45.7	1100	169.3	late vitellogenesis	3
hatchery produced	high	03/11/2016	24.3	120	0.2	perinucleolar	2
		03/11/2016	25.0	150	0.4	perinucleolar	2
		03/11/2016	25.2	150	0.3	perinucleolar	2
		03/11/2016	26.6	170	0.3	perinucleolar	2
		03/11/2016	26.9	160	0.3	perinucleolar	2
		03/11/2016	34.6	370	1.0	perinucleolar	2
		03/11/2016	36.4	430	1.1	perinucleolar	2
		03/11/2016	39.1	510	3.3	perinucleolar	2
		13/09/2017	29.3	180	0.4	perinucleolar	3
		13/09/2017	33.7	370	1.1	perinucleolar	3
		13/09/2017	35.1	360	0.7	perinucleolar	3
		13/09/2017	37.2	530	69.5	late vitellogenesis	3
		13/09/2017	42.1	780	159.2	late vitellogenesis	3
		13/09/2017	48.2	1143	114.0	late vitellogenesis	3
hatchery produced		03/11/2016	47.1	1240	128.2	100% atretic follicles	6
		03/11/2016	50.3	1480	8.1	perinucleolar	6
		03/11/2016	54.4	1970	34.4	perinucleolar	6
		03/11/2016*	41.2	750.0	0.6		6
		03/11/2016*	45.2	1130.0	2.8		6

Asterisk: male specimens used only for age and growth analyses.

Results

Biometric data analysis

No statistical difference was observed in body length and mass between captive-reared and hatchery-produced grey mullet of the same age class (**Fig. 2**) and no statistically significant effect of rearing density on fish body length and mass was found (**Figs. 3 and 4**).

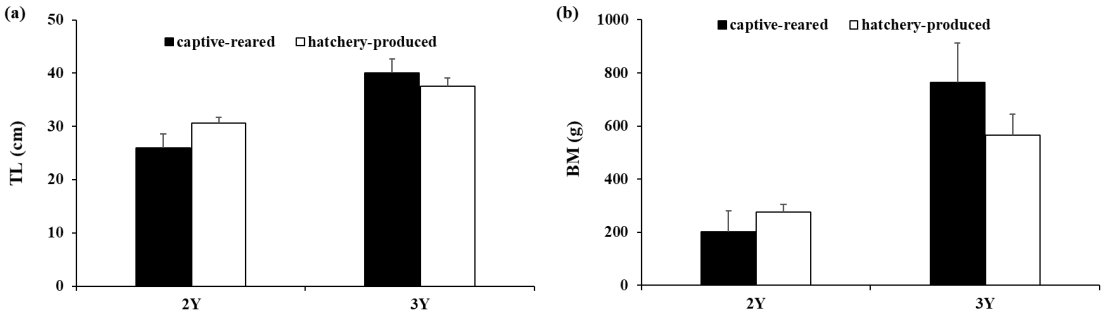

Fig. 2. Total length (TL) and body mass (BM) comparison between captive-reared and hatchery-produced grey mullet. 2Y, age 2; 3Y, age 3.

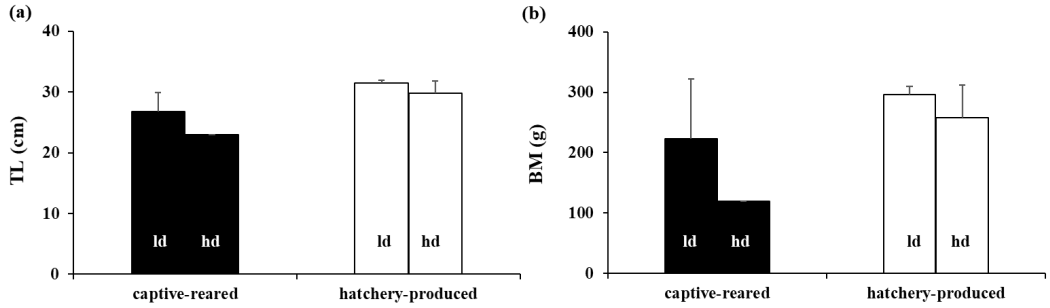

Figure 3. Total length (TL) and body mass (BM) comparison between age 2 grey mullet reared at low (ld) and high (hd) density.

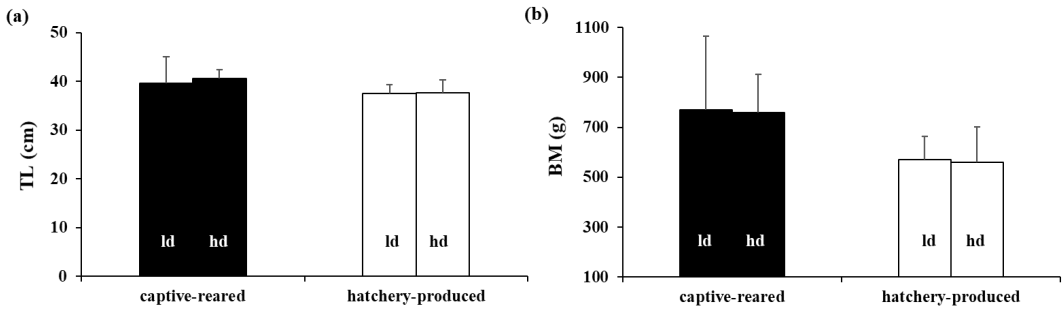

Figure 4. Total length (TL) and body mass (BM) comparison between age 3 grey mullet reared at low (ld) and high (hd) density.

Statistical differences in Fulton's condition factor, which is indicative of the overall fish nutritional state, were observed neither between captive-reared and hatchery-produced specimens (**Fig. 5**) nor between grey mullet reared at low and high density (**Fig. 6**).

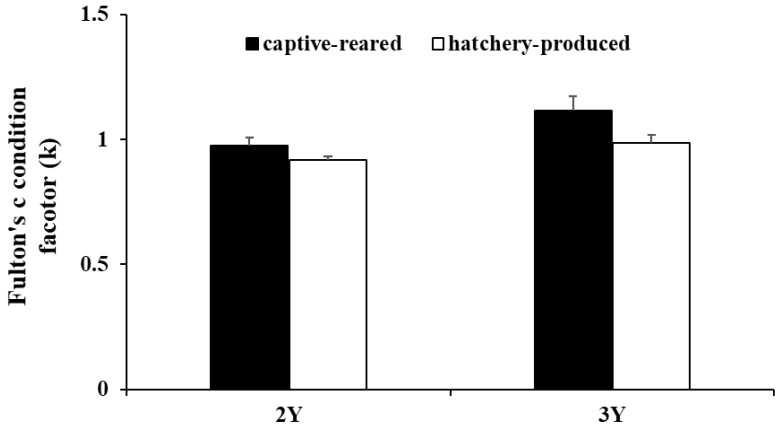

Fig. 5. Comparison of Fulton's condition factor between captive-reared and hatchery-produced grey mullet. 2Y, age 2; 3Y, age 3.

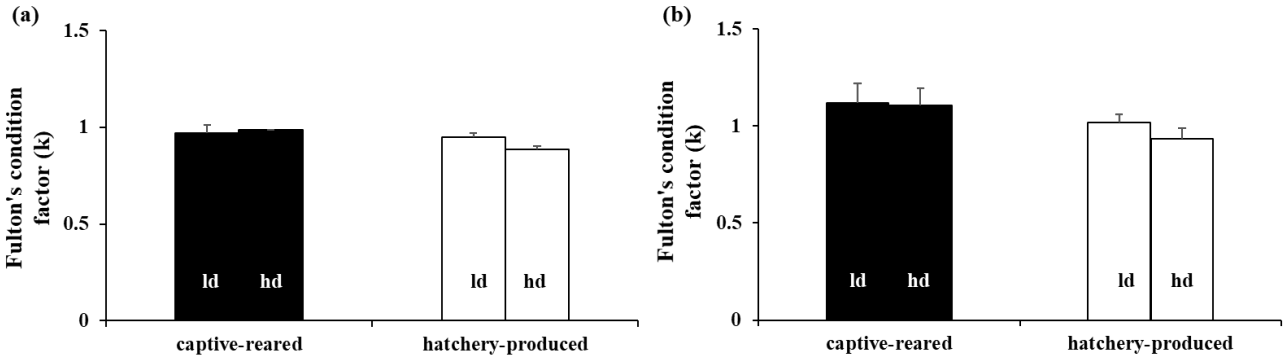

Fig. 6. Comparison of Fulton's condition factor between grey mullet reared at low (ld) and high (hd) density. (a) age 2; (b) age 3.

Age estimate and growth

According to the age estimate carried out on the basis of the number of annuli counted on their scales, among the 16 adult grey mullet sampled in the Lesina Lagoon, six belonged to the age class 4, seven to the age calls 5 and three to the age class 6 (**Table 1**).

Estimate of grey mullet theoretical growth in length was obtained by fitting the von Bertalanffy growth model (Bertalanffy von, 1938) to the mean lengths at estimated age. The obtained von Bertalanffy parameters were: TL_∞ = 64.08 cm; k = -0.230; t_0 = -0.001 (**Fig. 2**).

As shown in **Figure 6**, the mean length of age 6 hatchery-produced grey mullets reared at IOLR perfectly overlapped that of wild specimens of the same age sampled in Italy, indicating that grey mullet reared in captivity achieved a body growth similar to that of wild specimens.

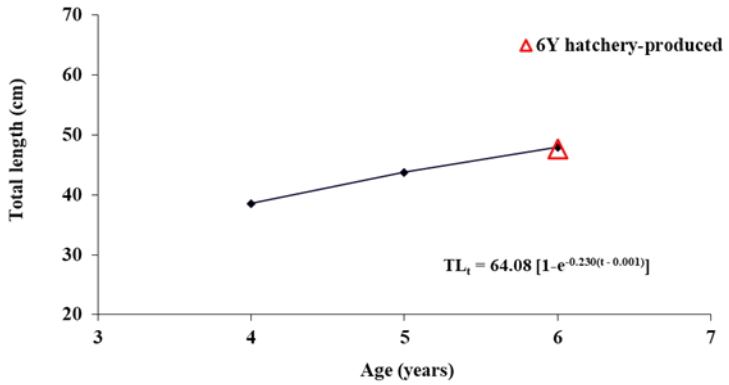

Figure 6. Von Bertalanffy growth curve of wild grey mullet. TL_t = predicted total length at age t. The red triangle refers to age 6 hatchery-produced specimens.

Morphological description of female germ cells

In the examined samples, oogonia along with the following oocyte developmental stages were observed: chromatin-nucleolus, perinucleolar, lipid, cortical alveoli, early vitellogenesis, late vitellogenesis (**Figs. 7 and 8**). Neither hydrated oocytes nor postovulatory follicles were found in any specimen.

Oogonia (**Fig. 7a**) (diameter 8-10 µm), often found in small clusters, were rounded cells with a large central euchromatic nucleus containing sparse eterochromatic patches.

Perinucleolar stage oocytes (**Fig. 7b, c**) (diameter 15-100 µm) were characterized by the presence of one or two large nucleoli centrally or eccentrically located in the nucleus and a variable number of small nucleoli adjoining the nuclear envelope. Early perinucleolar oocytes were rounded in shape and showed a strong ooplasm basophily. Late perinucleolar stage oocytes showed a reduced ooplams basophily and a variable polyedric shape. Flat follicular cells surrounded oocytes at this stage.

Oocytes at lipid/cortical alveoli stage (**Fig. 7d**) (diameter 100-150 µm) showed a further reduction of ooplasm basophily, numerous cortical alveoli in the peripheral ooplasm, many small lipid droplets in the inner ooplasm and the appearance of a thin zona radiata.

Secondary growth oocytes. Early vitellogenic oocytes (**Fig. 8a**) (diameter 150-300 µm) were characterized by the appearance of small eosinophilic yolk globules in the peripheral ooplasm and a further increase of the zona radiata thickness. Follicular cells surrounding oocytes at this stage became cubic. Late vitellogenic oocytes (**Fig. 8b**) (diameter 300-500 µm) showed an increase of the quantity and size of yolk granules, numerous lipid droplets amongst yolk globules and a ticker zona radiata.

Alpha atretic vitellogenic follicles displayed zona radiata fragmentation, coalescence of yolk globule and nucleus disintegration; in beta atretic follicles zona radiata and yolk globules were completely reabsorbed (**Fig. 8c**).

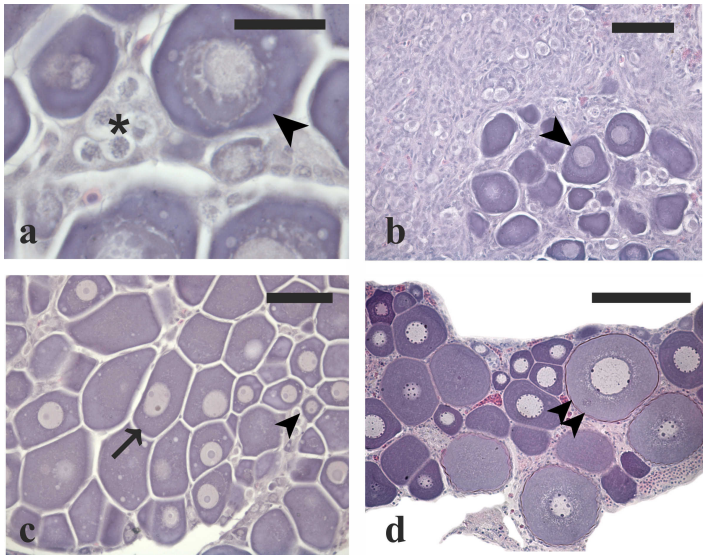

Figure 7. Micrographs of grey mullet ovary sections showing oogonia and oocytes in different developmental stages. a) and b) Oogonia (asterisk) and early perinucleolar stage oocytes (arrowhead). c) Early (arrowhead) and late (arrow) perinucleolar stage oocytes. d) Lipid stage oocyte (double arrowhead). Haematoxylin-eosin staining. Magnification bars = 20 µm in (a), 50 µm in (b)-(c), 150 µm in (d).

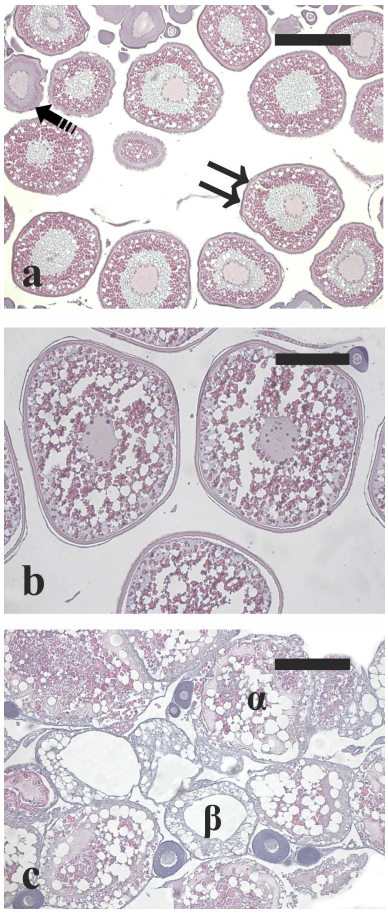

Figure 8. Micrographs of grey mullet ovary sections. a) Oocytes at cortical alveoli (dashed arrow) and early vitellogenic (double arrow) stage. b) Oocytes at late vitellogenic stage. c) α and β atretic vitellogenic follicles. Haematoxylin-eosin staining. Magnification bars = 200 µm.

Seasonal trend of ovarian development in wild grey mullet

Ovaries of wild grey mullet sampled in two different locations of the Mediterranean Sea showed a progressive development from May to September (**Table 1**): fish sampled in Divari lagoon or adjacent areas of the Ionian Sea showed only primary growth oocytes (perinucleolar/lipd stage oocytes as most advanced oocyte stage) during May-early July and early vitellogenic oocytes in late July. Grey mullet specimens sampled in Divari and Lesina lagoons showed late vitellogenic oocytes from late August to mid-September. No specimens in spawning conditions was observed throughout the investigate period in any of the two sampling areas.

Gonado-somatic index changed according to the oocyte maturation stage, showing stable values from May to July and a marked increase in August and September (**Fig. 9**).

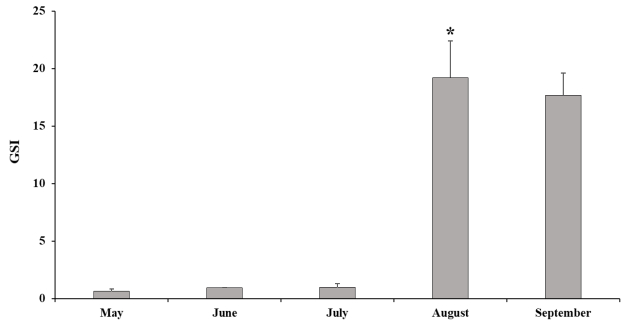

Figure 9. Gonado-somatic index of wild grey mullet sampled from May to September in different areas of the Mediterranean Sea. Asterisk: significant difference compared to the previous month ($P < 0.05$).

Reproductive state of hatchery-produced and captive-reared grey mullet.

All the age 2 grey mullet reared at IOLR were reproductively inactive, showing perinucleolar oocytes as the most advanced oocyte stage (**Table 2**). Statistically significant differences in GSI were observed neither between hatchery-produced and captive-reared specimens nor between fish reared at low and high density (**Fig. 10**).

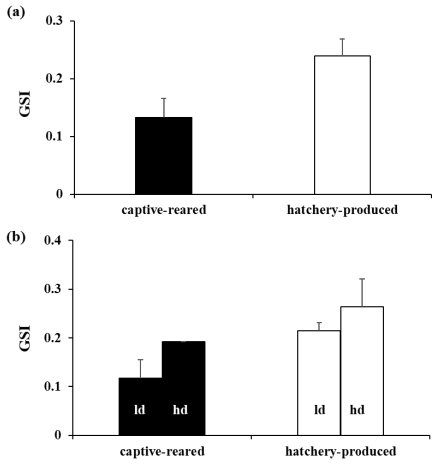

Figure 10. Gonado-somatic index (GSI) comparison between age 2 captive-reared and hatchery-produced grey mullet (a) and between age 2 grey mullet reared at low (ld) and high (hd) density (b).

Among the 20 age 3 specimens reared at IOLR, 11 were immature showing perinucleolar oocytes as the most advanced oocyte stage and 9 were reproductively active showing oocytes at late vitellogenic stage (**Table 2**). Among the 9 reproductively active specimens, 7 belonged to the hatchery-produced group and only 2 to the captive-reared group. Hatchery-produced specimens showed more than 3 times higher GSI than captive-reared fish (**Fig. 11**), although statistical significance was not reached due to the high variance.

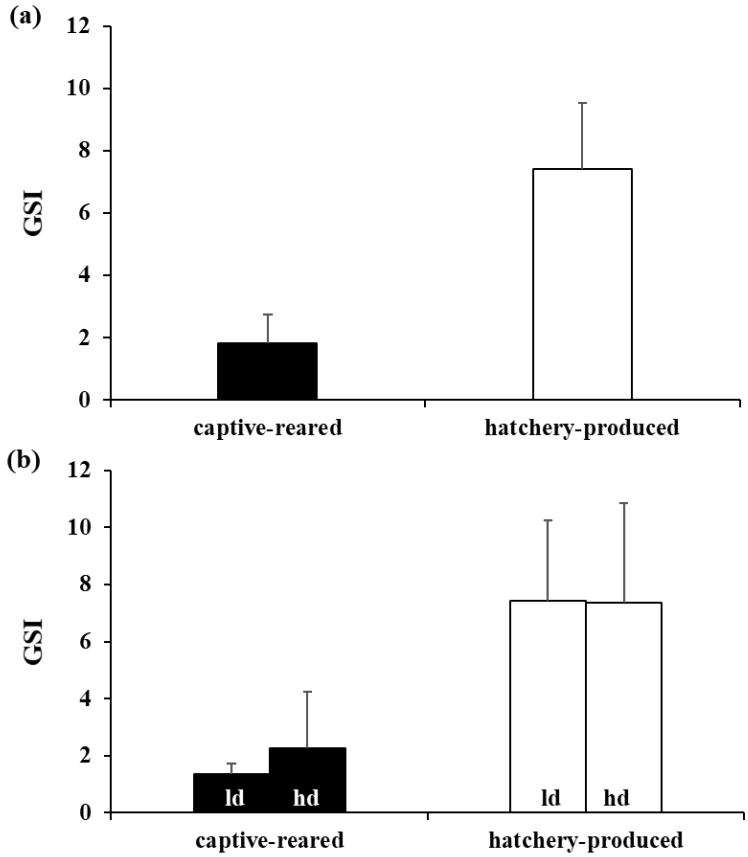

Figure 11. Gonado-somatic index (GSI) comparison between age 3 captive-reared and hatchery-produced grey mullet (a) and between age 3 grey mullet reared at low (ld) and high (hd) density (b).

Oocyte diameter and oocyte yolk accumulation

The mean diameter of the largest vitellogenic oocytes of age 3 hatchery-produced specimens was significantly higher than that of captive-reared fish and both farmed groups had significantly lower oocyte diameter than wild adults caught during their reproductive migration from the Lesina lagoon towards the open sea (**Fig. 12a**).

The surface occupied by yolk granules in oocytes of hatchery-produced grey mullet was higher than that of captive-reared specimens and it did not differ from that of wild adults caught during their reproductive migration from the Lesina lagoon towards the open sea (**Fig. 12b**).

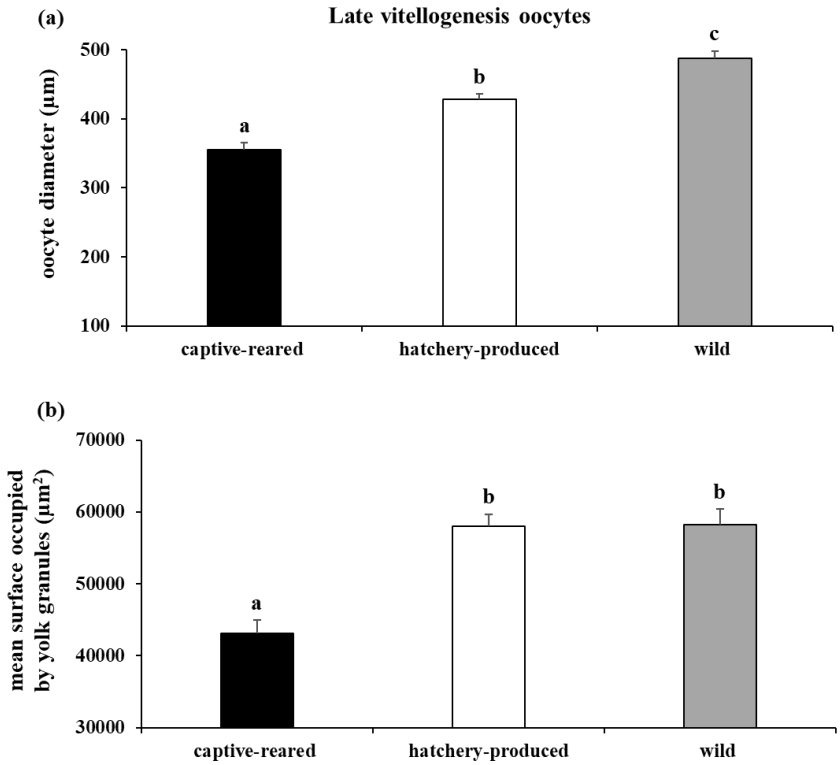

Figure 12. (a) Mean diameter of late vitellogenic oocytes and (b) mean surface occupied by yolk granules in oocytes of age 3 captive-reared and hatchery-produced grey mullets, and of wild adult females. Letters above bars indicate statistically significant differences ($P < 0.05$).

Discussion and Conclusions

The aim of the present work was to assess the effects of captivity on body growth, as well as on ovarian development and first sexual maturity on captive-reared and hatchery-produced grey mullet. This study was based on the analyses of: a) biometric data; b) age and growth; c) GSI and microscopic appearance of the ovaries.

The analysis of the biometric data did not show any significant effect of fish origin and rearing density on the body growth of grey mullet reared for 2 and 3 years in captivity. In fact, fish caught from the wild and reared in captivity had the same body size, mass and condition index of hatchery-produced fish. Similarly, no difference was observed in body size, mass and condition index between fish reared at two different densities.

A universal and validated method for grey mullet age estimation does not exist in the literature; however, scales (Tung, 1959, 1981; Hamza, 1999) and otoliths (Smith and Deguara, 2003) have been reported to be useful structures to estimate grey mullet age and growth. Limited data, mainly reported in grey literature, are available on grey mullet growth and they testify a rapid growth in the first year, with fish usually attaining 140–180 mm standard length (SL) in tropical and subtropical waters (Thomson, 1963; Wallace and van der Elst, 1975), and 130–160 mm SL in more temperate regions (Whitfield and Kok, 1992). Hendricks (1961) recorded a maximum size of 62 cm FL and Wallace (1975a) recorded similar sized specimens of 68–72 cm TL in the subtropical waters of Lake St Lucia. Thomson (1951, 1966) reported, for grey mullets captured in

temperate western Australian waters 38, 46 and 51 cm fork length (FL) at age 4, 5 and 6, respectively. In the present study, the age of 16 wild grey mullet captured during their migration from the Lesina lagoon to the Adriatic Sea was determined and the predicted mean TL at age 4, 5 and 6, calculated by the von Bertalanffy equation, was 39.0, 44.3 and 48.5 cm, respectively. Using the FL-TL correlation provided by Guino-o II (2012), these length-at-age data respectively correspond to 34.2, 38.2 and 41.8 cm FL. These data seem to indicate marked differences in the growth rate of grey mullet from the Adriatic Sea compared with that of the temperate western Australian waters. This difference is not unexpected and may be attributable to variations in growth within the *M. cephalus* species complex, to different environmental conditions as well as to the method used for the age determination. Interestingly, the mean length of 6 years old grey mullet caught from the wild at an early stage and reared in captivity at IOLR coincided well with that of wild specimens captured in the Lesina lagoon, indicating that the rearing conditions allowed fish to grow at a similar rate of the wild population.

The study of the temporal trend of GSI and ovarian microscopic appearance of wild grey mullet sampled in different areas of the Mediterranean Sea showed a progressive gonadal development from April to August-September. Histologically, none of the ovaries showed signs of imminent (oocytes in final maturation) or recent (post-ovulatory follicles) spawning and late vitellogenesis was the most advanced oocyte stage observed in the analysed ovary samples. These findings seem to confirm previous studies indicating a late summer-early autumn spawning period from the wild grey mullet from the Mediterranean Sea (Assem et al., 2008; Bartulović et al., 2011). The occurrence of specimens with high GSI and fully vitellogenic ovaries captured during their migration from the Lesina lagoon to the Adriatic Sea confirms that the ovary ripening process (i.e. vitellogenesis) occurs in estuarine/brackish waters and then the fish move to sea waters to spawn (Thomson, 1955; Ibáñez and Gutiérrez-Benítez, 2004).

In the present study, oocytes in late vitellogenesis were found in wild grey mullet having a minimum size of 38 cm TL. This body length, according to the TL-SL correlation provided by Guino-o II (2012) corresponds to 30 cm SL and falls within the first maturity size range reported by Ameur et al. (2003), i.e. 27–35 cm SL, for the grey mullet from the eastern Atlantic Ocean (Moroccan coasts).

The histological analysis of the ovaries demonstrated that farmed grey mullet started to be reproductively active at the age of 3 years. In particular, 54% (7/13) of age 3 hatchery-produced grey mullets were sexually mature vs 25% (1/4) of captive-reared fish. This finding seems to indicate that hatchery-produced grey mullet have a good reproductive potential, as they were able to attain sexual maturity at the same age of the wild population (Bok, 1983; Ameur et al., 2003).

The analysis of GSI and oocyte diameter clearly confirmed that age 3 hatchery-produced specimens attained a more advanced ovarian development than fish caught from the wild and reared in captivity. Similarly, the amount of yolk of oocytes from age 3 hatchery-produced fish was similar to that of wild adults and higher than that of captive-reared fish.

In conclusion, the present study indicates that: 1) the rearing condition established at IORL allows a growth rate equivalent to that of the wild grey mullet population from the Mediterranean Sea; 2) the reduction of the rearing density from 90 to 45 fish/m^3 has no effect on grey mullet growth and sexual maturity; 2) hatchery-produced grey mullet has a good potential to develop ovaries spontaneously up to a condition useful for bottarga production (advanced vitellogenesis).

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