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Reproductive management of the mugilid *Liza aurata* and characterization of proximate and fatty acid composition of broodstock tissues and spawnings

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ABSTRACT

The golden grey mullet (*Liza aurata*) is a promising species for aquaculture's sustainable expansion. However, the lack of sustainable juvenile provision, mainly related to the lack of reproductive control, is one of the most significant bottlenecks for further expanding the culture of this species. In many cases, mullet broodstock management needs the application of hormone treatments to induce gonadal maturation or spawning. However, no works are directly related to the broodstock collection, acclimation, and reproductive management of *Liza aurata*. On the other hand, the knowledge of essential fatty acids (EFA) requirements and mobilization patterns by the broodstock is a first step to designing appropriate feeding protocols and formulas, which are crucial for the success of reproduction and larval development.

For these reasons, this study aimed 1) to describe for the first time the reproductive management of *Liza aurata* broodstock under controlled conditions and 2) to offer a first approach to the reproductive lipid metabolism of the former species.

A selection of 22 *Liza aurata* broodstock from wild origin was acclimated in open seawater conditions. Additionally, the proximate and fatty acid composition of body tissues (gonads, liver, and muscle) of the initial wild population were evaluated, to be later compared with the profile of the eggs obtained after one year from the selected broodstock, for the first time described under cultured conditions.

The results highlighted the feasibility for the obtention of natural spawnings from broodstock with a mean weight of 787 g and 604 g (females and males, respectively), at a sex ratio of 2:1 (females/males), under natural photoperiod and marine water conditions with temperatures decreasing from 20.4 ± 0.3 °C to 18.8 ± 0.4 °C.

On the other hand, it was evidenced the crucial role of HUFA (highly unsaturated fatty acids) precursors for the gonadal development of *Liza aurata*, primarily for females. Additionally, the wild males' gonads presented a remarkable high content of HUFA, predominantly DHA (docosahexaenoic acid) (34% of the total fatty acids). In the eggs, significant variations appeared under captivity conditions, with lower levels of ARA (arachidonic acid) and EPA (eicosapentaenoic acid) and higher EPA/ARA and DHA/ARA ratios than the wild female gonad. Additionally, it was evidenced the significant role of the liver as a physiological reservoir of HUFA, which seem to be mobilized to the gonad during the maturation process.

Present results may help obtain a better insight to adjust broodstock management conditions and feeds, contributing to a more sustainable aquaculture growth.

1. Introduction

Aquaculture's diversification has been considered for the last decades as one of the major tools for a greater and more sustainable expansion of the sector (Abellán and Basurco, 1999). Among the different fish species considered for aquaculture diversification, mullets (Family Mugilidae) are great candidates due to their euryhaline, eurythermal, and opportunistic feeding nature, which have propitiated its extensive culture for centuries in different regions worldwide. However, its cultivation is supported traditionally, and still today, by wild fry capture, which

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produces high mortalities (from 70% to 96%), and, therefore, may be an unsustainable practice in the short term (Crosetti and Blaber, 2016). Despite fry collection being regulated in some of the countries involved, the lack of reproductive control is one of the most significant bottlenecks for further expanding the culture of these species.

Mullets generally have separate sex with no sexual dysmorphism, external fertilization and development. Females have group-synchronous ovaries (Wallace and Selman, 1981), being total or partial spawners, following the assignment of Hunter (1992). The golden grey mullet (*Liza aurata*) is one of the most abundant mullet species on the Atlantic coast and Mediterranean (Crosetti and Blaber, 2016). Despite having a smaller adult size than other mugilids, it has a great performance in the early stages (Hotos and Avramidou, 2020; Monzon et al., unpublished results), a slender body than other mullet species and a great fillet quality (Quirós-Pozo et al., 2021), making it an interesting choice for the development of its culture. However, the knowledge about reproductive characteristics is still very scarce, being the basic physiological and metabolic mechanisms unknown.

In this regard, the success in establishing new species is directly related to the controlled production of good quality eggs and larvae, which guarantee an adequate supply of juveniles (Izquierdo et al., 2001). Among the different parameters affecting egg quality, proximate composition, and especially, fatty acid profile are determinant factors affecting larval growth, survival, and early development of organs and tissues (Izquierdo and Koven, 2011, in Roo et al., 2015).

In this sense, broodstock nutrition not only affects the chemical composition of the eggs but also is one of the most significant factors affecting the success of the reproductive process (Fernández-Palacios et al., 2011). Particularly, dietary n3- HUFA affects ovarian steroidogenesis and vitellogenesis by altering FSH and LH levels in fish (Peng et al., 2015). Specifically, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) play an essential role in maintaining the structure and function of cell membranes, being the DHA the main component of glycerophospholipids in marine fish eggs (Izquierdo, 1996). Furthermore, arachidonic acid (ARA, 20:4n-6) derivates also participate in several reproductive functions in fish, being involved in processes like pheromonal attraction (Stacey and Sorensen, 2006), steroidogenesis (Henrotte et al., 2011; Norberg et al., 2017), fecundity, hatching rate and yolk sac diameter (Røjbek et al., 2014; Asil et al., 2017; Stuart et al., 2018). Besides the optimal ratios between these nutrients are crucial in broodstock diets since DHA/EPA and EPA/ARA compete for the same enzymes, affecting egg and sperm quality (Fernández-Palacios et al., 2011; Estefanell et al., 2015). Fish eggs should contain all nutrients necessary for embryonic and early larval development, so their biochemical composition can be a great indicator to develop adequate feeds for broodstock in captivity conditions (Izquierdo et al., 2001). Besides, the body composition of wild fish in different reproductive stages may shed some clues on their nutritional requirements (Pérez et al., 2007).

For these reasons, this study aimed to describe the reproductive management which allowed the obtention of viable eggs of this species and to evaluate the biochemical composition and fatty acid profile of wild mature and immature *Liza aurata* broodstock tissues and the eggs obtained under culture conditions. Present results will help to define criteria for better management by producers and allow the formulation of more adequate diets for this species.

2. Material and methods

All the procedures with animals were rigorously conducted according to the European Union Directive (2010/63/EU) on animal welfare protection for scientific purposes and were carried out in coordination between the GIA-Ecoaqua institute (Canary Islands, Spain) and the aquaculture facilities of the CIFP Zaporito (San Fernando, Cádiz, Spain).

2.1. Broodstock collection and management

The experiment lasted for over one year, including fishing, sampling, broodstock acclimation and finally, the successful obtention of natural spawnings in captivity. In September 2019, 125 golden grey mullets (*Liza aurata*) were captured from a semi-natural estuary in San Fernando, Cádiz (Spain) (36° 27′ 56″ N; 6° 11′ 48″ W), where they were fed just from the natural food available.

After capture, fish were transferred to the aquaculture facilities of the CIFP Zaporito and sampled to be individually pit-tagged and biopsied, to determine the size, weight, sex ratio, and state of sexual maturation (Table 1). For the latter, fish were anesthetized with clove oil (40 ppm) and abdominal massage was applied to identify mature males. Negative animals for this test were gonadally biopsied with a 1.3-mm-internal-diameter catheter (Kruuse, Langeskov, Denmark). The sampled material was stored in Serra's fixative (6: 3: 1, 70% ethanol, 40% formaldehyde and 99.5% acetic acid) to later view under the microscope (148 LED Digital Zuzi®). The diameter of biggest oocytes (n = 30) was measured with the software TS View Zuzi®.

22 fish were selected as broodstock and acclimated in a separated tank of 10 m3 for one year, in an open seawater system under natural photoperiod (36° 27′ 56″ N; 6° 11′ 48″ W). Fish were fed once per day (0.5% of biomass day^{-1}) with a commercial extruded feed (R5 Europe, Skretting). To calculate the growth of the selected broodstock along the experimental period (Table 2), the following formulas were used:

$$\text{Weight gain (g)} = \text{final weight (g)} - \text{initial weight (g)}$$

$$\text{Weight gain (\%)} = (\text{final weight (g)–initial weight (g)}) \text{ x } 100/\text{initial weight}$$

$$\text{Specific growth ratio (SGR) (\%)} = (ln \text{ final weight}–ln \text{ initial weight})/\text{days of experiment x100}$$

2.2. Samplings of broodstock tissues and eggs

From the initial population (2019), four mature females, four mature males, and four immature mullets were sacrificed by clove oil overdose.

Table 1
Medium values of weight, length and somatic indexes in females, males and immature specimens of *Liza aurata* collected from the estuary.

	Mature females	Mature males	Immature
Weight (g)	475.00 ± 49.45^{a}	321.38 ± 66.74^{b}	280 ± 78.01^{b}
Lenght (cm)	38.80 ± 2.20^{a}	34.54 ± 3.02^{b}	30.98 ± 3.66^{c}
Population percentage (%)	8	10.4	81.6
HSI (%)	0.98 ± 0.19	0.94 ± 0.27	0.61 ± 0.07
GSI (%)	9.75 ± 1.40^{a}	2.53 ± 1.10^{b}	–

Data expressed as means ± SD (standard deviation). Values with different superscripts in the same row indicate the presence of significant differences ($p \leq 0.05$).

Table 2
Weight, length, and growth parameters of *Liza aurata* broodstock along the experimental period.

	Females	Males
Initial weight (g)	424.62 ± 74.54	352.75 ± 35.05
Initial lenght (cm)	37.23 ± 3.75	35.88 ± 1.36
Final weight (g)	787.40 ± 76.05	604.40 ± 48.64
Final lenght (cm)	38.55 ± 2.49	36.40 ± 2.72
Weight gain (g)	362.78	251.65
Weight gain (%)	85.43	71.34
SGR (%)	0.14	0.12

Data of weight and length expressed as means ± SD.

Table 3
Proximate composition in dry weight of different tissues of females, males and immature specimens of *Liza aurata* collected from the estuary.

	Gonad			Muscle			Liver
	Lipids	Protein	Ash	Lipids	Protein	Ash	Lipids
Females	32.88 ± 1.13^{a}	62.30 ± 2.30^{b}	3.31 ± 0.31^{b}	4.46 ± 1.88	90.88 ± 8.97	7.48 ± 0.58	20.67 ± 1.75^{b}
Males	24.49 ± 2.81^{b}	86.27 ± 4.68^{a}	7.67 ± 0.80^{a}	9.41 ± 1.39	90.77 ± 4.95	7.17 ± 0.42	22.73 ± 1.57^{ab}
Immature	-	–	–	6.23 ± 1.81	93.12 ± 4.33	7.69 ± 0.92	24.63 ± 1.42^{a}

Data expressed as means ± SD. Values with different superscripts in the same column indicate the presence of significant differences ($p \leq 0.05$).

Samples of muscle, liver, and gonads were collected for histological, biochemical, and gonadal development analyses. Gonado-somatic and hepato-somatic indexes (GSI and HSI) (Table 1) were calculated according to the following formulas:

$$\text{HSI}\ (\%) = \text{liver weight}\ (g)/\text{fish weight}\ (g) \times 100$$

$$\text{GSI}\ (\%) = \text{gonad weight}\ (g)/\text{fish weight}\ (g) \times 100$$

Samples of eggs from 5 different natural spawnings collected from October to November 2020 were also saved for biochemical analyses.

2.3. Biochemical and fatty acid analyses

Protein, moisture, and ash content of fish tissues (Table 3) and eggs (Table 5) were determined by the techniques described in AOAC (2000). Total lipids were quantified following the method described by Folch et al. (1957). For fatty acid determination (tissues, eggs, and commercial diet) (Tables 4 and 6), the total lipids were trans-esterified (Christie, 1989), and the fatty acids obtained were quantified by gas chromatography (Izquierdo et al., 1992).

2.4. Histological analysis

Gonadal samples from the initial population were fixed in 4% formalin and then dehydrated by graded ethanol series and placed in paraffin blocks. Thin layers (4 μm) were made using a microtome (AUTOCUT JUNG 2055, LEICA, Lyon, France), and the sections were placed in slides and stained following the haematoxylin and eosin (H&E) technique (Martoja and Martoja-Pierson, 1970). The histological evaluation was carried out by direct visualization to determine the maturation state of the fish according to the criteria described by Genten et al. (2009) and by González-Castro et al. (2011).

2.5. Statistical analyses

The statistical analyses were carried out using the program IBM®

Table 4
Fatty acid profile, expressed in % of the total fatty acids identified, of gonads, liver and muscle of wild females, males and immature specimens of *Liza aurata* collected from the estuary.

Gonads			Liver			Muscle		
	Females	Males	Females	Males	Immature	Females	Males	Immature
Saturated	15.36 ± 1.23	15.52 ± 1.68	34.29 ± 5.55	29.60 ± 2.27	29.03 ± 1.74	27.96 ± 2.39	22.62 ± 3.40	29.52 ± 5.03
Monoenoics	42.05 ± 1.76^{a}	17.57 ± 2.04^{b}	28.79 ± 3.62^{a}	28.01 ± 3.48^{a}	17.05 ± 3.55^{b}	38.23 ± 4.02^{ab}	40.86 ± 5.19^{a}	30.02 ± 3.48^{b}
n-3	24.16 ± 3.69^{b}	54.28 ± 3.81^{a}	22.11 ± 6.01^{b}	28.28 ± 2.20^{b}	39.57 ± 3.76^{a}	15.02 ± 1.38	18.44 ± 6.86	21.89 ± 6.38
n-6	16.24 ± 1.27^{a}	11.45 ± 1.38^{b}	13.70 ± 1.74	12.79 ± 1.03	12.96 ± 1.29	16.08 ± 2.11	15.76 ± 0.73	15.96 ± 1.02
n-9	27.00 ± 0.72^{a}	12.29 ± 1.57^{b}	17.55 ± 3.06^{a}	16.21 ± 2.34^{a}	9.20 ± 2.52^{b}	27.47 ± 1.13^{ab}	28.34 ± 0.42^{a}	21.02 ± 3.94^{b}
n3 PUFA	23.44.34 ± 3.75^{b}	53.34 ± 4.13^{a}	21.86 ± 6.01^{b}	28.03 ± 2.20^{b}	39.35 ± 3.75^{a}	13.95 ± 1.71	17.83 ± 6.81	21.06 ± 6.60
n6 PUFA	16.24 ± 1.27^{a}	11.43 ± 1.39^{b}	13.70 ± 1.74	12.79 ± 1.03	12.95 ± 1.30	16.06 ± 2.10	15.74 ± 0.73	15.94 ± 1.02
Total PUFA	41.16 ± 2.66^{b}	65.55 ± 2.93^{a}	36.13 ± 5.80^{b}	41.48 ± 2.46^{b}	52.89 ± 3.34^{a}	31.01 ± 1.35	34.56 ± 7.29	37.65 ± 7.41
14:0	0.76 ± 0.18a	0.30 ± 0.30b	0.88 ± 0.28	0.99 ± 0.64	0.98 ± 0.52	1.05 ± 0.63	1.39 ± 0.45	1.49 ± 0.68
16:0	10.65 ± 0.82	9.76 ± 1.86	21.80 ± 4.10	19.45 ± 3.56	17.26 ± 1.77	18.15 ± 1.66	15.06 ± 3.33	19.23 ± 3.15
16:1n-7	10.03 ± 1.81^{a}	1.39 ± 0.54^{b}	3.57 ± 0.32	4.13 ± 1.67	2.80 ± 0.34	4.65 ± 2.34	5.42 ± 1.22	4.55 ± 1.01
18:0	3.65 ± 0.30	4.99 ± 1.06	11.04 ± 1.36	9.83 ± 1.45	10.25 ± 0.46	8.16 ± 3.54	5.39 ± 0.62	7.96 ± 2.32
18:1n-9	25.80 ± 0.79^{a}	11.53 ± 1.38^{b}	16.93 ± 2.94^{a}	16.10 ± 2.76^{a}	8.72 ± 2.44^{b}	26.08 ± 1,17^{ab}	26.89 ± 3.84^{a}	19.98 ± 3,68^{b}
18:1n-7	4.68 ± 0.08^{a}	2.15 ± 0.28^{b}	5.14 ± 0.26^{a}	4.87 ± 0.46^{a}	3.29 ± 0.79 ^{b}	3.8 ± 0.32^{a}	4.03 ± 0.59^{a}	2.80 ± 0.21^{b}
18:2n-6	12.20 ± 1.12^{a}	3.66 ± 0.53^{b}	8.02 ± 1.10^{a}	6.99 ± 2.07^{ab}	4.25 ± 1.38^{b}	9.94 ± 0.46^{a}	10.30 ± 1.08^{a}	6.57 ± 1.60^{b}
18:3n-3	1.33 ± 0.24^{a}	0.56 ± 0.20^{b}	0.67 ± 0.22	0.89 ± 0.26	0.59 ± 0.11	0.97 ± 0.38	1.17 ± 0.25	0.76 ± 0.13
20:1n-9	0.07 ± 0.20	0.07 ± 0.02	0.13 ± 0.09	0.17 ± 0.04	0.06 ± 0.02	0.15 ± 0.06	0.21 ± 0.08	0.14 ± 0.04
ARA 20:4n-6	1.35 ± 0.17^{b}	3.87 ± 0.94^{a}	3.55 ± 0.66	3.34 ± 1.34	4.66 ± 0.80	3.43 ± 1.79^{ab}	2.38 ± 0.66^{b}	4.81 ± 0.60^{a}
EPA 20:5n-3	3.75 ± 0.26^{b}	10.28 ± 2.25^{a}	4.43 ± 0.74^{b}	4.47 ± 1.50^{b}	8.86 ± 2.13^{a}	3.48 ± 0.36	4.21 ± 1.68	6.82 ± 2.44
DHA 22:6n-3	12.90 ± 4.08^{b}	33.96 ± 5.53^{a}	13.86 ± 5.20^{b}	16.60 ± 6.36^{ab}	24.37 ± 3.27^{a}	6.65 ± 1.04	8.61 ± 3.80	9.19 ± 2.87
DPA 22:5n-6	0.71 ± 0.12^{b}	1.91 ± 0.40^{a}	0.65 ± 0.14^{b}	0.79 ± 0.38^{ab}	1.45 ± 0.52^{a}	0.88 ± 0.36	1.11 ± 0.56	2.17 ± 0.92
DHA/22:5n-6	18.03 ± 4.17	18.76 ± 5.91	21.64 ± 7.10	22.35 ± 8.86	19.02 ± 8.44	8.98 ± 5.17	7.99 ± 1.40	4.71 ± 2.06
EPA/ARA	2.79 ± 0.26	2.75 ± 0.58	1.26 ± 0.23	1.40 ± 0.44	1.91 ± 0.34	1.31 ± 0.83	1.77 ± 0.37	1.41 ± 0.45
DHA/EPA	3.44 ± 1.35	3.30 ± 0.89	3.13 ± 0.80	3.82 ± 0.58	2.86 ± 0.78	1.92 ± 0.32	2.03 ± 0.48	1.38 ± 0.31
DHA/ARA	9.54 ± 3.30	8.78 ± 3.17	3.90 ± 1.48	5.53 ± 1.91	5.42 ± 1.50	2.70 ± 2.18	3.59 ± 1.01	1.92 ± 0.63
Oleic/DHA	2.13 ± 0.53^{a}	0.35 ± 0.08^{b}	1.33 ± 0.49^{a}	1.22 ± 0.90^{ab}	0.37 ± 0.13^{b}	3.98 ± 0.56	3.75 ± 1.97	2.44 ± 1.17
Oleic/n3 PUFA	1.12 ± 0.18^{a}	0.22 ± 0.03^{b}	0.81 ± 0.25^{a}	0.58 ± 0.09^{a}	0.23 ± 0.08^{b}	1.89 ± 0.21	1.71 ± 0.73	1.06 ± 0.50
n-3/n-6	1.49 ± 0.36^{b}	4.74 ± 0.86^{a}	1.61 ± 0.55^{b}	2.22 ± 0.25^{ab}	3.08 ± 0.49^{a}	0.95 ± 0.19	1.16 ± 0.39	1.36 ± 0.34

Data expressed as means± SD. Values with different superscripts in the same row indicate significant differences ($p \leq 0.05$). Contains 14:1n-7, 14:1n-5, 15:0, 15:1n-5, 16:OISO, 16:1n-5, 16:2n-4, 16:3n-4, 16:3n-3, 16:3n-1, 16:4n-3, 17:0, 18:1n-5, 18:2n-9, 18:2n-4, 18:3n-6, 18:3n-4, 18:3n-1, 18:4n-3, 18:4n-1, 20:0, 20:1n-7, 20:1n-5, 20:2n-9, 20:2n-6, 20:3n-9, 20:3n-6, 20:3n-3, 20:4n-3, 22:1n-11, 22:1n-9, 22:4n-6 and 22:5n-3.

SPSS Statistic 20 (New York, USA). Homogeneity of variances was performed using Levene's test ($p \geq 0.05$). To analyze the variance, a one-way ANOVA was used, being the means compared by Tukey post-hoc test ($p \leq 0.05$). In the cases where the data did not meet normality or homoscedasticity, the medians were compared using a non-parametric test (Kruskal Wallis) ($p \leq 0.05$).

3. Results

3.1. Broodstock collection and management

The average weight and length of the sampled population, as well as GSI and HSI from sacrificed animals are presented in Table 1. The percentage of mature females from the total identified (oocyte diameter ≥ 600 μm) was 39%, being their weight and length significantly higher than males and immature animals ($p \leq 0.05$). For the 22 fish selected as broodstock, both females and males almost double their weight in one year (Table 2), being the final sex ratio of 2:1 (females/males). Under these conditions, viable spawns were obtained from late September to late November of 2020 when temperature were decreasing from 20.4 ± 0.3 °C to 18.8 ± 0.4 °C.

3.2. Biochemical and fatty acid analyses

The proximate composition of the gonad from the initial population (Table 3) showed sex related differences. The lipid content in the ovaries was higher than in the testicles; on the other hand, protein and ash were lower in ovaries. In addition, females presented a lower percentage of hepatic lipids than immature animals.

The fatty acid composition of gonads, liver, and muscle (Table 4) also presented clear differences among groups. The gonad of initial mature females had higher levels of linolenic (LNA, 18:3n-3), linoleic (LA, 18:2n-6), monoenoic, n6, and n9 fatty acids than males. In comparison, initial male's gonads presented higher ARA, n3, and n3 PUFA (polyunsaturated fatty acids), particularly EPA and DHA, than female gonads, which caused an increase in the total PUFA and n3/n6 ratio.

In the liver, minor differences between sexes were found; however, in comparison with mature animals, immature ones presented lower levels of n9 fatty acids, oleic acid (18,1n-9), and oleic/n3 PUFA ratio, and higher levels of EPA, n3, n3 PUFA and total PUFA. In addition, levels of DHA and DPA (docosapentaenoic acid), were higher in immature animals than in females. Levels of n-6 were similar in all groups.

In the muscle, few differences between groups were found in the fatty acid composition. LA was higher in mature animals than in immature ones. ARA showed the highest levels in immature mullets, followed by females. EPA, DHA, DPA, total PUFA, n3, n6 fatty acids, and ratio n3/n6 were similar in all groups, the last one close to 1.

Regarding biochemical results of eggs obtained after one year in captivity (Table 5), they presented higher lipid content ($p \leq 0.05$) than the wild female gonad. The fatty acid composition of the eggs (Table 6), reflected in many cases the profile of the diet with some exceptions as the content of EPA, LNA, EPA/ARA and n3/n6 ratios, which were lower in the eggs, or the content of DHA and DHA/EPA ratio which were higher than those of the diet. The eggs fatty acid profile also showed many similarities with the wild female gonad, with some exceptions as the levels of ARA and EPA which were higher in the wild female gonad ($p \leq 0.05$). HUFA ratios also were quite different to those of the wild female gonad, with higher values of both EPA/ARA and DHA/ARA ratios for the eggs ($p \leq 0.05$).

Table 5
Proximate composition in dry weight of *Liza aurata* broodstock commercial diet and eggs.

Proximate composition (%)	Lipids	Protein	Ash
Diets	20	52	8.3
Eggs	39.15 ± 4.18	56.91 ± 9.24	4.23 ± 3.36

Data of the commercial diet obtained from the product label. Data of eggs is expressed as means ± SD.

Table 6
Fatty acid profile, expressed in % of the total fatty acids identified, of the commercial diet given to *Liza aurata* broodstock, and *Liza aurata* spawnings after one year in captivity.

	Diet	Eggs
Saturated	16.43	16.15 ± 0.67
Monoenoics	44.97	45.79 ± 0.99
n-3	22.52[a]	18.54 ± 0.93[b]
n-6	15.32	17.31 ± 0.90
n-9	29.12[b]	32.66 ± 0.94[a]
n3PUFA	22.15[a]	18.24 ± 0.77[b]
n6PUFA	15.05	17.07 ± 0.88
Total PUFA	43.59[a]	37.36 ± 1.00[b]
14:0	1.61	1.07 ± 0.32
16:0	10.67	11.31 ± 0.47
16:1n-7	2.90[b]	7.64 ± 1.09[a]
18:0	3.35	3.52 ± 0.26
18:1n-9	27.76	30.85 ± 1.08
18:1n-7	2.74	5.39 ± 0.96
18:2n-6	12.93	14.96 ± 0.75
18:3n-3	4.05[a]	2.46 ± 0.31[b]
20:1n-9	0.78[a]	0.10 ± 0.04[b]
ARA 20:4n-6	0.60	0.58 ± 0.09
EPA 20:5n-3	6.09[a]	2.33 ± 0.47[b]
DHA 22:6n-3	8.37[b]	9.83 ± 0.33[a]
DPA 22:5n-6	0.26	0.23 ± 0.02
DHA/22:5n-6	32.04	44.02 ± 4.27
EPA/ARA	10.09[a]	4.03 ± 0.47[b]
DHA/EPA	1.37[b]	4.35 ± 0.81[a]
DHA/ARA	13.87	17.33 ± 2.19
Oleic/DHA	3.32	3.14 ± 0.12
Oleic/n3 PUFA	1.25[b]	1.98 ± 0.12[a]
n-3/n-6	1.47[a]	1.07 ± 0.07[b]

Data expressed as means ± SD. Values with different superscripts in the same row indicate significant differences ($p \leq 0.05$). Contains 14:1n-7, 14:1n-5, 15:0, 15:1n-5, 16:OISO, 16:1n-5, 16:2n-4, 16:3n-4, 16:3n-3, 16:3n-1, 16:4n-3, 17:0, 18:1n-5, 18:2n-9, 18:2n-4, 18:3n-6, 18:3n-4, 18:3n-1, 18:4n-3, 18:4n-1, 20:0, 20:1n-7, 20:1n-5, 20:2n-9, 20:2n-6, 20:3n-9, 20:3n-6, 20:3n-3, 20:4n-3, 22:1n-11, 22:1n-9, 22:4n-6 and 22:5n-3.

3.3. Histological analysis

Regarding the histological examination of the gonads (Fig. 1), the ovary of all females considered for the study showed a typical structure of fully mature spawners, with a synchronous group of larger oocytes full of vitellogenin next to a stock of previtellogenic oocytes. The male gonads were also fully mature, presenting testicular cysts full of spermatozoa.

4. Discussion

The obtained results of gonadal maturation and egg production identify the spawning season of *Liza aurata* in the north-eastern Atlantic region between September and November, which is in concordance with reported data from Hotos et al. (2000) for this species in the Mediterranean or by Ghaninejad et al. (2010) in the Caspian Sea, this last highlighting that the spawning is promoted by temperatures decreasing from 20 to 22 °C.

The average weight and length of mature females and males from the initial population sampled (475 g, 39 cm and 321 g, 35 cm, respectively) are quite similar to those reported by Fazli et al. (2008). The length reported in our study is higher than the length of first sexual maturity reported by Fehri Bedoui et al. (2002), who described an L50 (the length in which 50% of the animals were mature) of 21 cm for males, and 22 cm for females of *Liza aurata* in Tunisian waters, and also by Kesiktaş et al. (2020), who reported a length of first sexual maturity of 26 cm for males

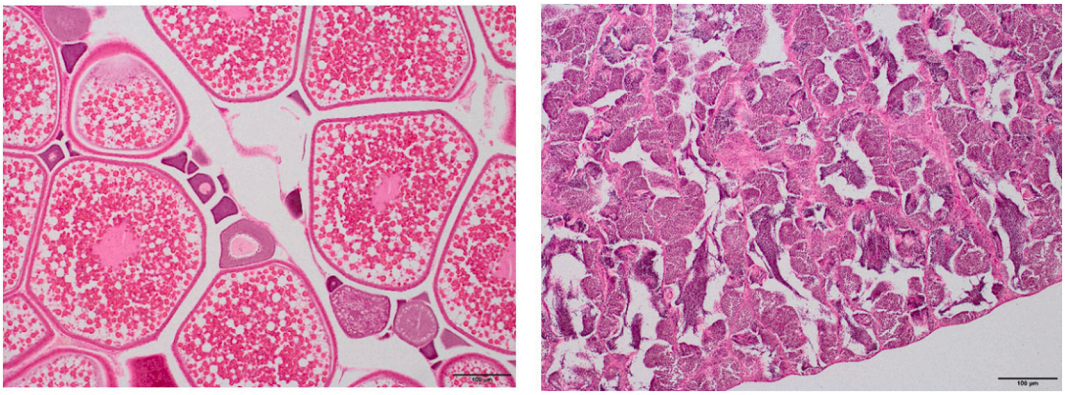

Fig. 1. Female (left) and male (right) gonads sections of mature *Liza aurata* specimens stained with H&E. Bars 100 μm.

and 24 cm for females. In this regard, present results suggest an adequate minimal weight for established wild collected *Liza aurata* broodstock above 300 g for males and 400 g for females.

Broodstock feeding with a commercial diet at 0.5% of biomass day^{-1}, resulted in fish almost doubling their weight in one year, with SGR values between 0.12 and 0.14 and successful natural spawns, indicating that this feeding dose seems to be adequate for broodstock maintenance. However, both dose and feeding frequency may be increased when the objective is the growth of the animals, as it has been described for mullet's juveniles (Calixto da Silva et al., 2020; Solovyev and Gisbert, 2022). Under the described conditions of salinity, temperature, and sex ratio, viable spawns have been obtained spontaneously, for first time described in captivity, which open new perspectives to increase the controlled culture of this species.

On the other hand, it has been widely described how the composition of the diet can be reflected in the fish tissues, especially in the gonads (Izquierdo et al., 2001; Jaya-Ram et al., 2008). As regards the evaluation of initial *Liza aurata* broodstock tissues, as the origin of the animals was the same, it can be supposed that the main differences in the lipids and fatty acids were primarily due to the specific physiological status of the animals. In general, for fish females, specific lipid mobilization patterns depend on whether the animals reduce their feed intake in the spawning season or not. In the first case, the body reserves supply the nutrients for ovary development (Aksnes et al., 1986; Lal and Singh, 1987). In species like the gilthead seabream (*Sparus aurata*), the Atlantic cod (*Gadus morhua*), or the smooth weakfish (*Cynoscion leiarchus*), which continue feeding during the spawning season, the lipids deposited in the ovary are both from the diet and the mobilization of the body reserves, mainly from the liver (Almansa et al., 2001; Dahle et al., 2003; do Carmo Silva et al., 2019). Based on the HSI and the condition factor (Kc), Fehri Bedoui et al. (2002) proposed that the golden grey mullet used both liver and muscle reserves to develop the gonads in both sexes. In the present study, the lipid content and fatty acid composition of liver was affected by the maturation state of the fish, being these differences less evident in the case of muscle. From the information listed above, it can be deduced that *Liza aurata* (primarily females) used both lipids from the diet and body reserves (including liver and muscle) to supply nutrients to the gonad during the maturation period.

In this study, the high levels of 18:1n-7, oleic acid and LA both in the wild female gonad and eggs reflected the importance of these fatty acids in the natural reproduction and embryonic processes of this species. Oleic acid has been described as a crucial fatty acid involved in gonadal maturation for both females and males of the white seabream (*Diplodus sargus*), increasing in the spawning season and decreasing after (Pérez et al., 2007). Also, in concordance with the previous study, in the present work the levels of oleic acid in the liver of both wild males and females were higher than those of immature animals, which confirm the role of the liver as a fatty acid reservoir to support the reproduction process along the spawning season. In the present study, also the amount of LA was high in both liver and muscle of mature specimens. This suggest that these fatty acids not only must be abundant in the natural diet of the fish under study but also that they have been selectively incorporated by mature animals to the tissues evaluated. LA levels were also markedly higher than those previously reported for other wild marine species (Pérez et al., 2007; Rodríguez-Barreto et al., 2012), however, they were like those reported for female gonads and eggs from fish maintained in captivity and fed with diets with certain contents of vegetable sources (Jaya-Ram et al., 2008; El-Husseiny et al., 2018; Ferosekhan et al., 2021).

Marine fish generally cannot transform 18C fatty acids into HUFA (Castro et al., 2012), so they must obtain these nutrients from the diet, however, it has been described how high availabilities of HUFA precursors can increase elongation and desaturation processes in some fish species (Ling et al., 2006; Jaya-Ram et al., 2008). In addition, it has been defined a certain capacity of HUFA biosynthesis for mullets (Garrido et al., 2019; Galindo et al., 2021), including fatty acyl desaturase 2 with Δ6 activity in *Liza aurata* (Mourente and Tocher, 1993). Also, it has been described for *Liza aurata* juveniles (Quirós-Pozo et al., 2021) higher levels of DHA and ARA in the whole body than those present in the diets, thus supporting the potential of this species to synthetize HUFA from its precursors. Moreover, the nutritional programming thought low FM and FO broodstock diets had been shown to influence HUFA biosynthesis metabolism in both parents and offspring, also affecting the posterior growth performance of the juveniles (Xu et al., 2019; Turkmen et al., 2019). Furthermore, increased dietary LNA/LA ratios produced higher plasma estradiol and gonadosomatic index in species with HUFA biosynthesis potential like the common carp (*Cyprinus carpio*) (Ma et al., 2020). This data supports the potential feasibility of Liza broodstock diets containing high levels of vegetable sources as an alternative to marine fish meal and oil ingredients, however, correct n3/n6 ratios may be taken under consideration.

On the other hand, male gonads showed a notary predominance of n-3 HUFA, primarily due to high levels of DHA, which is in concordance with its recognized influence on the reproductive process and fertility (Mansour et al., 2011). In different species such as the rainbow trout (*Oncorhynchus mykiss*) or the seabass (*Dicentrarchus labrax*), (Izquierdo et al., 2001), the sperm fatty acid composition depends on the essential fatty acid content of the broodstock diet. The levels of DHA in the sperm of *Liza aurata* males (34 ± 6%) were higher than those reported in other fish species like the Arctic char (*Salvelinus alpinus*) (26–24%) (Mansour et al., 2011), the sterlet (*Acipenser ruthenus*) (14%) (Engel et al., 2020), the wild white seabream (12.24%, calculated data) (Pérez et al., 2007), the gilthead seabream (*Sparus aurata*) (10.5%, calculated data) (Martín et al., 2009), or the rainbow trout (20%) (Vassallo-Agius et al., 2001), suggesting both the critical role of DHA content in *Liza aurata* sperm, the meaningful natural intake that the animals under study may have had in

the estuary, or even the probable biosynthesis of this fatty acid from precursors of the diet. Also, ARA is remarkably higher for male gonads than for females, in concordance with its key role for fish sperm motility and therefore for the ability to fertilize the eggs (Butts et al., 2015; Kowalski and Cejko, 2019).

The low percentage of EPA, DHA, n3, and n3 PUFA in the liver of mature animals in comparison with immature ones, may be explained by mobilization from the liver to the gonad in the first case, which highlights the role and selective mobilization of those fatty acids for the maturation process.

Selective retention of EFA, primarily DHA, has been described for different fish eggs (Izquierdo et al., 2001; Fernández-Palacios et al., 2011). This is in concordance with the present results, in which although the eggs reflected the fatty acid profile of the diet in many cases, the levels of EPA were quite lower in the eggs, while DHA levels were higher, showing a preferential conservancy of DHA compared to EPA. Additionally, the eggs presented lower levels of EPA, ARA, and a lower n3/n6 ratio than the wild female gonad, which may indicate a nutritional disbalance of the commercial diet for these animals. Therefore, until more studies could be done on the potential use of high HUFA precursors levels for this species' diets, ARA and EPA supplementation is recommended to achieve similar amounts in the eggs than in the wild female gonad.

As regards the histological evaluation of the mature gonads of *Liza aurata*, these animals presented similar features than those reported for other mullet species like de thin-lipped grey mullet (*Liza ramada*) or the grey mullet (*Mugil cephalus*) (Chang et al., 1995; Mousa et al., 2018). According to the recommended classification of Crosetti and Blaber (2016), the stage of ovarian maturity of the females under study was the IV stage (advanced maturity), in agreement with the categorization of González-Castro et al. (2011) for the "*tainha*" (*Mugil platanus*).

To summarize, *Liza aurata* seems to be a promising species for aquaculture diversification due to its feasible reproductive management in captivity, which may lead to viable egg production in only one year of acclimation under the present conditions. Furthermore, the present findings highlight that *Liza aurata* diets in nature may include important quantities of vegetable sources, opening the possibility of utilizing high levels of vegetable ingredients in *Liza aurata* broodstock diets as an alternative to marine fish meal and oil, although more studies are needed to evaluate this potential for reproductive and larval performance. Thus, the present findings contribute to more sustainable aquaculture while open also clear possibilities for the circular economy close to this species' production sites.

CRediT authorship contribution statement

Raquel Quirós-Pozo: Conceptualization, Investigation, Writing – original draft. **Lidia Robaina:** Conceptualization, Writing – review & editing, Funding acquisition. **Juan Antonio Calderón:** Investigation. **Javier Roo Filgueira:** Supervision, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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