Lipid and fatty acid composition of muscle, liver, ovary, and peritoneal fat in wild flathead grey mullet (*Mugil cephalus*) according to ovarian development

Sandra Ramos-Júdez [a]*, Alicia Estévez, Wendy Ángela González-López and Neil Duncan*

IRTA, Sant Carles de la Ràpita Ctra. de Poble Nou km. 5.5, 43540 Sant Carles de la Ràpita, Tarragona, Spain.

[a]Present address: S2AQUAcoLAB, Av. Do Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal

* Corresponding authors:

Sandra Ramos-Júdez, Email: sandra.ramos@s2aquacolab.pt

Neil Duncan, Email: neil.duncan@irta.cat

Abstract

Wild adult females of a low trophic omnivore teleost species, the flathead grey mullet (*Mugil cephalus*), caught in the western Mediterranean were sampled. The lipid and fatty acid composition of ovaries, liver, muscle, and peritoneal fat were analysed at previtellogenesis, early-vitellogenesis —first observed at mid-summer (early August)—, late-vitellogenesis, and the post-spawning period —from mid-September to mid-October—. During ovarian development, the lipid content of muscle was low and constant (3.85% - 4.92%), indicating that the muscle was not used to store lipids for gonadal growth. Although constant, lipid content in the liver was higher (18.46% - 22.62%) than in the muscle, and HSI% increased during gonad development, suggesting a dynamism in the mobilization of the hepatic lipids. Total lipids in the gonads significantly increased with maturation (from 4.90% to 34.59%) in parallel with the GSI (from 0.8% to 15.5%) to decrease after spawning. Peritoneal fat was probably transitional fat that could be rapidly metabolized or transferred to other tissues but no specific function could be assigned because its presence in previtellogenic and early-vitellogenic females varied greatly. One of the main sources of lipids accumulated in the ovary was most likely diet. The total percentage of ΣMUFA, mainly 17:1 —previously not identified in high quantities in teleost vitellogenic ovaries and likely of bacterial origin— and 16:1, strongly increased in the ovaries with maturation. The 16:1 might be an important source of lipids for embryo development. High percentages of DHA, EPA, and ARA were found in the ovary during previtellogenesis available to be used during gonadal maturation. Understanding lipid and fatty acid changes in broodstock tissues can increase our knowledge of the nutritional requirements of the fish used in aquaculture breeding programs.

Keywords: *Mugil cephalus*, western Mediterranean, fatty acids, liver, muscle, ovary

1. Introduction

The flathead grey mullet (*Mugil cephalus*) has a cosmopolitan world distribution and high human consumption demand in the Mediterranean region, Asia, and the United States of America [1]. This mugilid fish can be cultured in a variety of salinities and presents high growth rates [2]. It is classified as a low-trophic level species due to its omnivorous diet [3]. Given the European Commission's recommendation for producing species with a lower environmental footprint [4] and the flathead grey mullet's positive market and culture attributes, this species could have a great potential for aquaculture diversification. Nevertheless, there are bottlenecks that limit the ability to scale up the industrial production, such as reproductive dysfunctions in captivity [5] and juvenile availability [6,7]. There is also a lack of knowledge on the seasonality of reproductive development in different regions and on the nutrient requirements of breeders, which implies that broodstock artificial feeds have not been developed yet. Mullets are typically fed on chicken manure, food leftovers, detritus, or pellets that target other species since are usually cultured in semi-intensive polyculture systems where they constitute less than the twenty percent of the stock [7].

Nutrition is important in reproductive development. The lipid and fatty acid composition of the broodstock diet has been recognized as the key metabolic energy resource that determines the successful reproduction and survival of offspring [8,9]. Some fatty acids, such as highly unsaturated fatty acids and particularly arachidonic acid (20:4n-6, ARA), eicosapentaenoic acid (20:5n-3, EPA), and docosahexaenoic acid (22:6n-3, DHA), are not only essential components of the gametes but also precursors of physiologically active molecules such as prostaglandins and other eicosanoids that are directly linked with reproductive development and success [9]. The use of inadequate diets that do not meet the nutritional requirements of breeders can have adverse effects in their reproductive

success [10–12]. A reduction in gamete fertilization, hatching, and/or larvae survival has been observed in a variety of fish species, including freshwater eels (*Anguilla spp.*) [13], Senegalese sole (*Solea senegalensis*) [14], common sole (*Solea solea*) [15], gilthead seabream (*Sparus aurata*) [16], Japanese flounder (*Paralichthys olivaceus*) [17], red sea bream (*Pagrus major*) [18], yellowtail seriola (*Seriola quinqueradiata*) greater amberjack (*Seriola dumerili*) [19], Atlantic halibut (*Hippoglossus hippoglossus*) [20], mangrove red snapper (*Lutjanus argentimaculatus*) [21] and lumpfish (*Cyclopterus lumpus*) [22]. Lipids can be acquired (i) directly from food, (ii) *de novo* synthesized in the gonads, or (iii) mobilized from storage tissues to the gonads [11]. Therefore, the lipid dynamics through the reproductive cycle are related to their functions [23]. To our knowledge, the changes in lipid and fatty acid mobilization and deposition related to reproduction in the flathead grey mullet have not been established. Furthermore, the spawning season in the western Mediterranean has not been determined, as it has been in the Eastern Mediterranean regions (Turkish, Egyptian, Greek, and Tunisian coasts), the Black Sea, the Aegean Sea, the Atlantic Ocean (USA, Mauritanian, and Moroccan coasts), the Gulf of Mexico, the Indian Ocean (Indian, Sri Lankan, and South African coasts), and the Pacific Ocean (Australian coast) [24–26], indicating the need for year-round evaluation.

In this regard, the main goal of this study was to describe the total lipid content and fatty acid composition of various tissues (muscle, liver, and ovaries) and peritoneal fat during different stages of ovarian development in the flathead grey mullet, an omnivore fish with an attractive low-trophic position. This research will provide a better understanding of the lipid requirements for this species' reproductive development, which will eventually enable the development of broodstock-specific diets.

2. Materials and methods

2.1. Fish samples

Wild flathead grey mullet female fish (n = 44) obtained in October and November 2018 and between February and October 2019 were sampled. Samples were not obtained during the winter time —December 2018 and January 2019— as no females were captured. Samples were collected and processed once a month (mid-month), except from July to October when samples were obtained twice a month (first and third weeks of the month). Fish were caught in the Ebro Delta canals (Spain) and the western Mediterranean (Subarea 37.1.1 of FAO) that comprises the waters to the north of the Ebro Delta, between the Spanish mainland and Sardinia —without including the north-western Gulf of Lion— , and were kept on ice during transport to the laboratory and while being processed. Each fish was measured (standard length, SL; fork length, FL; and total length, TL) to the nearest 0.5 cm and weighted to the nearest 1 g with an electronic balance (Cobos Precision, Spain). Whole liver and ovary weights were also recorded to the nearest 0.1 g (Mettler Toledo, Spain). Two condition indices, the gonadosomatic index (GSI) and the hepatosomatic index (HSI), common metrics of reproductive allocation and reproductive condition in fisheries biology, were estimated as follows: (Wg or Wl / W) x 100, where Wg and Wl correspond to the gonad and liver weights respectively, and W to the body weight. Peritoneal fat (fat around the peritoneal cavity) weight was also recorded. Sections of the ovaries were taken from the anterior, middle, and posterior parts of the right and left lobes and preserved in a Bouin's solution for later histological examination.

For lipid and fatty acid analysis, ~5-g samples of gonads, liver, muscle from directly under the dorsal fin, and peritoneal fat were collected. Each sample was stored at -20ºC until further analysis. A total of 27 flathead grey mullet females were selected at four different phases of the reproductive cycle for lipid and fatty acid analysis; at

previtellogenesis (n = 7; 1637 ± 359 g BW; 46.7 ± 3.28 cm SL), early-vitellogenesis (n = 6; 2522 ± 437 g; 52.6 ± 3,3 cm), late-vitellogenesis (n = 7; 2435 ± 477 g; 50.6 ± 3.9 cm), and in the post-spawn period (n = 7; 2256 ± 520 g; 52.9 ± 5.2 cm).

2.2. Lipid content and fatty acid analysis

Total lipids were extracted from the samples by homogenization in chloroform/methanol (2:1, v:v) according to the method of Folch *et al*. [26], using a double extraction, and were quantified gravimetrically after evaporation of the solvent under a nitrogen flow followed by vacuum desiccation overnight. Total lipids were stored in chloroform:methanol (2:1) containing 0.01% butylated hydroxytoluene (BHT) at -20 ºC before fatty acid transmethylation. Fatty acids were methylated following the acid-catalyzed transmethylation method used by Christie [27]. Methyl esters were extracted twice using isohexane diethyl ether (1:1, v:v), purified on TLC silica plates (Macherey-Nagel, Düren, Germany) and quantified through gas-liquid chromatography analysis on a Thermo TraceGC (Thermo Fisher, Spain) fitted with a Thermo TR-FAME capillary column (30 m × 0.25 mm id; Thermo Scientific, Spain), using a two-stage thermal gradient from 50 ºC (injection temperature) to 150 °C after ramping at 40 ºC min^{-1} and holding at 250 ºC after ramping at 2 ºC min^{-1}. Helium (1.2 mL min^{-1} constant flow rate) was used as the carrier gas, and on-column injection and flame ionization detection at 250 ºC were used. Peaks of each fatty acid were identified and quantified according to the response to the internal standard, 21:0 fatty acid (Sigma-Aldrich, Spain, Ref. H5149), added before transmethylation.

To obtain the percentage of total lipids, ~200 mg of each sample was weighted to the nearest 0.01 mg (KERN & SOHN GmbH, Germany) before and after being dried at 100ºC (Memmert, Germany) for 24h. The water percentage was calculated to obtain the dry

139 weight of samples. Total lipid percentages were obtained by dividing the total lipid weight
140 by the dry weight of each sample multiplied by 100. An estimation of the total fatty acids
141 accumulated in the entire gonad was made by calculating the quantity of fatty acids (µg)
142 per milligram of humid tissue sample multiplied by the total weight (mg) of the fresh
143 gonad.

144

145 **2.3. Histological analysis**

146 Ovarian samples were dehydrated in gradually increasing ethanol (76 % to 96 %) and
147 xylene solutions and embedded in paraffin. Three-µm thick sections were cut, stained
148 with hematoxylin and eosin and examined under a light microscope (Leica DMLB,
149 Houston, USA). The histological classification of flathead grey mullet ovaries followed
150 the description of Greeley *et al*. [29]. The ovaries were classified based on the ovary's
151 most advanced oocyte stage. Previtellogenic ovaries contained small oocytes in
152 perinucleolar stages (typically without yolk or lipid droplets), early-vitellogenic ovaries
153 contained a clutch of vitellogenic oocytes as well as a heterogeneous clutch of smaller
154 oocytes, and late-vitellogenic ovaries contained a clutch of large vitellogenic oocytes and
155 a clutch of previtellogenic oocytes. Females were identified as post-spawn when their
156 ovaries contained postovulatory follicles (POFs).

157

158 **2.8. Statistical analysis**

159 Data are presented as mean ± standard deviation (SD) unless otherwise noted. All
160 variables were checked for normal distribution with the Shapiro-Wilk test and
161 homogeneity of variances with the Levene test. Total lipid (%) data was 1/x transformed,
162 and GSI % was transformed by square root to follow normality. Data were analysed by

one-way analysis of variance (ANOVA) to determine differences between different stages of gonadal development, followed by Holm Sidak's multiple comparisons. No statistical analysis was performed in total lipids (%) and fatty acids from peritoneal fat as it was obtained from only four and one females at previtellogenesis and early-vitellogenesis, respectively. All statistical analyses were performed using SigmaPlot v12.0 (Systat Software Inc., Richmond, CA, USA). Significance was set at $P < 0.05$. The power of the performed ANOVAs identifying significant differences was high, with values greater than 0.8.

3. Results

3.1. Biometric data

A total of 44 females were collected and stages of ovary development were determined; 18 females were at previtellogenesis, six females were at early-vitellogenesis, 13 females were at late-vitellogenesis, and seven were at post-spawning period (Table 1). Previtellogenic females were obtained from mid-October 2018 to early August 2019, had GSIs ≤ 1 %, and small pink ovaries (Fig 1A) filled with previtellogenic oocytes (Fig 2A). Females at early-vitellogenesis were collected from early August to early September and had slightly higher GSIs; however, this time, the ovaries turned to yellowish pink (Fig 1B) because of the recruitment of oocytes into yolk stages in the ovaries (Fig 2B). Females at late-vitellogenesis were collected from mid-August, together with some females at early-vitellogenesis, to mid-September. Females at late-vitellogenesis had significantly larger ovaries represented by a GSI value of 15.5 ± 3.4 % (ranging from 11 to 22.6 %) ($P < 0.001$). These ovaries had a yellow to orange colour (Fig 1C) due to the abundance of oocytes at late-vitellogenesis stage (Fig 2C). Females with opaque pink colour ovaries with a red hue from extensive vascularization (Fig 1D) were also obtained

in the mid of September and through to mid-October. These females presented POFs (Fig 2D) indicating post-spawning period, together with variable atresia and a batch of previtellogenic oocytes. The GSI values in this post-spawn ovary presented a sharp decrease to 1.8 ± 0.9 %.

Table 1. Biometric data of *Mugil cephalus* (n = 44) captured in the western Mediterranean. Values are mean ± SD. Values with different superscripts within rows are statistically different.

Morphometry	Maturation stage			
	Previtellogenesis (n = 18)	Early-vitellogenesis (n = 6)	Late-vitellogenesis (n = 13)	Post-spawning (n = 7)
Body weight (g)	1591.7 ± 423.9^{a}	2355.1 ± 515.8^{b}	2261.4 ± 377.2^{b}	2256.5 ± 520.7^{b}
Standard length (cm)	46.1 ± 4.4^{a}	51.2 ± 4.9^{a}	47.28 ± 2.7^{a}	52.9 ± 5.2^{a}
Fork length (cm)	50.2 ± 4.7^{a}	53.3 ± 5.4^{ab}	51.2 ± 3.1^{a}	57.6 ± 5.6^{b}
Total length (cm)	53.9 ± 6.0^{a}	59.6 ± 5.0^{ab}	55.6 ± 3.4^{a}	62.4 ± 5.9^{b}
Gonadosomatic index (GSI %)	0.8 ± 0.3^{a}	2.7 ± 1.1^{a}	15.5 ± 3.4^{b}	1.8 ± 0.9^{a}

3.2. Total lipid content in tissues at different ovarian development

The lipid content in the muscle, liver, and ovaries of female flathead grey mullet breeders ranged from 3.85 % - 4.92 %, 18.46 % - 22.62 %, and 4.90 % - 34.59 %, respectively, between groups in different gonadal stages (Fig 3). During ovarian development, total lipid content in the muscle and liver did not differ significantly. However, HSI % was significantly higher ($P = 0.003$) during vitellogenesis and at a post-spawning period compared to previtellogenesis (Fig 4). As the ovaries of flathead grey mullet developed, the total lipid content in the ovaries changed significantly ($P < 0.001$) with the lowest values obtained at previtellogenesis. There was a significant increase ($P < 0.001$) through

vitellogenesis with a peak in late-vitellogenesis and a significant decrease (P = 0.014) at the post-spawning period. The lipid accumulation in the gonads followed the same pattern of GSI % (Table 1, Fig 4), which significantly increased (P < 0.001) from previtellogenesis (0.8 %) to late-vitellogenesis (15.5 %) and decreased afterwards (1.8 %). Total lipid percentage increased 7-fold and GSI increased 19-fold that led to an increase of 133-fold in the lipid content of the entire ovaries. Forty-five % of females (eight out of 18) that were at previtellogenesis and 17 % (one out of six) that were at early-vitellogenesis, presented peritoneal fat whereas no fish had peritoneal fat during late vitellogenesis (n = 13) or post-spawning (n = 7). The proportion of fish in previtellogenesis that presented peritoneal fat was similar from February to August, while the only female with peritoneal fat in early-vitellogenesis was from early August. The total lipid content in peritoneal fat of those fish selected for lipid and fatty acid analysis was 75.81 ± 13.97 % at previtellogenesis (4 out of 7 females) and 95.95 % at early-vitellogenesis (1 out of six females) (Fig 3).

3.3. Fatty acid composition at different ovarian developmental stages in different tissues

3.3.1. Muscle and peritoneal fat

In general terms, the fatty acid composition was stable in the muscle with little variation during ovarian development. Only 22:5n-3 percentage presented a significant decrease (P = 0.002) through ovarian development and during the post-spawning period. The ARA/EPA ratio significantly increased at late-vitellogenesis and post-spawning (Table 2).

231 Peritoneal fat from individuals at previtellogenesis (four out of seven females) and early-
232 vitellogenesis (one out of six females) was mainly composed of 16:0, 16:1 and n-3 PUFA
233 (Table 3). Peritoneal fat was completely absent in all but one fish that presented
234 vitellogenic oocytes.
235
236 **3.3.2. Liver**
237 As the ovaries developed from previtellogenesis to late-vitellogenesis, the percentage of
238 ΣSFA in the liver showed a significant increase (P = 0.02) (Fig 5, Table 4). The
239 percentage of Σn-3 PUFA significantly decreased (P = 0.024) from previtellogenesis to
240 late-vitellogenesis due to a decrease in EPA, 22:5n-3 and DHA percentages. The
241 reduction of EPA percentages was significant (P = 0.001) at late-vitellogenesis and after
242 spawning and, thus, it affected the DHA/EPA and ARA/EPA ratios that increased
243 accordingly (Table 4).
244
245 **3.3.3. Gonad**
246 During ovarian development, an enormous gradual accumulation of fatty acids was
247 observed in the entire ovary. From previtellogenesis to late-vitellogenesis, the estimated
248 total quantity in the entire ovary of the most representative fatty acids groups (ΣSFA,
249 ΣMUFA, Σn-6 PUFA, ARA, Σn-3 PUFA, EPA, DHA and ΣPUFA) increased
250 significantly (Table 5). However, there were both increases and decreases in the fatty acid
251 balance. The percentage of ΣSFA significantly decreased from previtellogenesis to late-
252 vitellogenesis and after spawning (P = 0.001) (Fig 5, Table 6). The most noteworthy
253 decrease in individual fatty acids during vitellogenesis was due to a downturn in 16:0 that
254 remained low after spawning. On the contrary, the percentage of ΣMUFA significantly
255 increased (P < 0.001) along vitellogenic development, mainly due to a rise in 16:1, 17:1,

18:1n-9, and 18:1n-7 fatty acids, and then decreased again after spawning. The most noticeable increases were in 16:1 and 17:1 content. The 16:1 fatty acid was not present in previtellogenic ovaries and appeared during vitellogenesis. Additionally, 17:1 rose approximately 5-fold in late-vitellogenesis in comparison with previtellogenesis. Total n-6 PUFA percentage significantly decreased ($P < 0.001$) during vitellogenesis and increased after spawning, following the same trend as ARA. The total n-3 PUFA percentage, including the major fatty acids 22:5n-3, EPA, and DHA, decreased during gonadal development. The percentages of 22:5n-3 and DHA increased again after spawning, while EPA values remained low after spawning. Therefore, the DHA/EPA and ARA/EPA ratios increased significantly during the post-spawning period.

Table 2. Fatty acid composition (% of total fatty acids) of flathead grey mullet female muscle at different maturation stages (n = 6 – 7 females per stage). Values are mean ± SD. Fatty acids with < 0.5 % are excluded. Values with different superscripts within rows indicate significant differences in fatty acid % between different maturation stages (previtellogenesis, early-vitellogenesis, late-vitellogenesis and post-spawning).

Fatty acid	Previtellogenesis	Early-vitellogenesis	Late-vitellogenesis	Post-spawning
14:0	1.57 ± 0.69	2.1 ± 1.36	1.81 ± 1.12	1.21 ± 0.99
15:0	0.95 ± 0.34	1.92 ± 1.6	3.67 ± 2.5	3.7 ± 2.89
16:0	22 ± 3.16	22.2 ± 3.23	21.17 ± 1.14	20.79 ± 3.67
18:0	7.21 ± 1.84	6.8 ± 2.47	7.85 ± 1.5	8.98 ± 1.75
ΣSFA	**31.93 ± 2.34**	**33.02 ± 2.67**	**34.69 ± 2.23**	**34.83 ± 4.06**
16:1	7.44 ± 4.78	9.77 ± 6.01	6.66 ± 3.32	5.11 ± 4.65
18:1n-9	4.02 ± 1.12	3.77 ± 1.28	4.74 ± 0.75	4.92 ± 1.5
18:1n-7	3.4 ± 0.65	3.33 ± 1.2	4.74 ± 1.31	4.55 ± 1.64
20:1	0.07 ± 0.09	0.03 ± 0.08	0.99 ± 2.05	0.13 ± 0.09
ΣMUFA	**15.09 ± 6.41**	**17.05 ± 6.8**	**16.37 ± 2.99**	**14.74 ± 7.15**
18:2n-6	1.07 ± 0.54	1.77 ± 1.23	0.68 ± 0.49	0.68 ± 0.6
18:3n-6	1.62 ± 0.93	1.78 ± 0.38	1.26 ± 0.38	1.05 ± 0.32
20:4n-6 ARA	7.34 ± 2.04	6.99 ± 1.38	7.75 ± 1.19	9.14 ± 2.64
22:4n-6	0.86 ± 0.25	0.72 ± 0.26	0.91 ± 0.22	0.97 ± 0.32
22:5n-6	1.5 ± 0.59	1.12 ± 0.4	1.73 ± 0.45	1.66 ± 0.63
Σn-6 PUFA	12.38 ± 2.07	12.38 ± 1.79	12.28 ± 1.7	13.49 ± 3.07
18:3n-3	0.4 ± 0.2	0.65 ± 0.52	0.28 ± 0.2	0.26 ± 0.12
18:4n-3	0.89 ± 0.59	1.37 ± 1.02	0.85 ± 0.64	0.6 ± 0.31
20:4n-3	0.66 ± 0.24	0.52 ± 0.22	0.33 ± 0.2	0.35 ± 0.21
20:5n-3 EPA	11.14 ± 2.32	10.83 ± 1.98	8.23 ± 2.08	8.73 ± 3.28
22:5n-3	7.34 ± 1.62^{a}	5.52 ± 1.33^{b}	4.85 ± 0.58^{b}	4.9 ± 1.01^{b}
22:6n-3 DHA	14.33 ± 4.77	11.79 ± 5.75	14.07 ± 3.49	13.53 ± 5.56
Σn-3 PUFA	35.13 ± 7.55	31.23 ± 5.36	28.8 ± 4.6	28.57 ± 7.63
ΣPUFA	**47.51 ± 8.57**	**43.61 ± 4.98**	**41.08 ± 5.57**	**42.06 ± 9.8**
DHA/EPA	1.27 ± 0.26	1.14 ± 0.62	1.79 ± 0.51	1.57 ± 0.42
ARA/EPA	0.66 ± 0.14^{a}	0.66 ± 0.19^{a}	0.99 ± 0.28^{b}	1.1 ± 0.23^{b}
Total FA (mg g^{-1} lipids)	521.64 ± 37.26	507.4 ± 91.57	485.3 ± 54.77	425.17 ± 81.76

Table 3. Fatty acid composition (% of total fatty acids) in flathead grey mullet female peritoneal fat at different maturation stages (n = 4 females at previtellogenesis and n = 1 at early-vitellogenesis). Values are mean ± SD. Fatty acids with < 0.5 % are excluded. No statistical analysis was performed.

Fatty acid	Previtellogenesis	Early-vitellogenesis
14:0	3.41 ± 0.81	1.20
15:0	1.08 ± 0.48	0.49
16:0	24.25 ± 2.69	26.59
18:0	3.27 ± 0.51	8.15
ΣSFA	**32.05 ± 3.25**	**36.42**
16:1	18.18 ± 2.19	7.47
18:1n-9	7.46 ± 1.28	9.92
18:1n-7	7.60 ± 1.13	9.71
ΣMUFA	**33.66 ± 0.52**	**27.48**
18:2n-6	1.89 ± 0.60	1.73
18:3n-6	1.22 ± 0.10	0.86
20:4n-6 ARA	2.13 ± 1.27	6.15
22:4n-6	0.49 ± 0.30	1.18
22:5n-6	0.40 ± 0.11	1.36
Σn-6 PUFA	6.13 ± 2.04	11.29
18:3n-3	1.14 ± 0.39	0.53
18:4n-3	3.23 ± 0.8	0.53
20:4n-3	1.76 ± 1.61	0.94
20:5n-3 EPA	6.94 ± 5.25	4.32
22:5n-3	3.78 ± 2.28	5.44
22:6n-3 DHA	4.04 ± 1.36	12.33
Σn-3 PUFA	24.72 ± 4.61	24.80
ΣPUFA	**30.85 ± 2.73**	**36.09**
Total FA (mg g^{-1} lipid)	863.27 ± 71.67	697.47

289 **Table 4. Fatty acid composition (% of total fatty acids) of flathead grey mullet**
290 **females liver at different maturation stages (n = 6 – 7 females per stage).** Values are
291 mean ± SD. Fatty acids with < 0.5 % are excluded. Values with different superscripts
292 within rows indicate significant differences in fatty acid % between different maturation
293 stages.
294

Fatty acid	Previtellogenesis	Early-vitellogenesis	Late-vitellogenesis	Post-spawning
14:0	0.75 ± 0.25	1.62 ± 0.51	1.12 ± 1.06	1.02 ± 0.48
15:0	0.82 ± 0.3	2.6 ± 3.14	2.65 ± 1.53	2.57 ± 1.36
16:0	20.48 ± 1.63	26.71 ± 5.43	24.33 ± 7.57	23.15 ± 8.11
18:0	8.23 ± 3.11	8.51 ± 3.27	11.68 ± 3.75	10.42 ± 3.35
ΣSFA	**30.64 ± 4.18^{a}**	**39.52 ± 5.91^{ab}**	**40.26 ± 5.58^{b}**	**37.75 ± 7.06^{ab}**
14:1	4.12 ± 5.8	11.21 ± 4.26	7.35 ± 5.34	7.25 ± 5.31
16:1	3.8 ± 3.84	1.85 ± 2.48	2.45 ± 1.71	2.22 ± 1.07
18:1n-9	4.52 ± 3.64	6.46 ± 3.39	7.3 ± 2.16	7.26 ± 4.93
18:1n-7	8.6 ± 4.32	7.62 ± 1.08	9.3 ± 3.13	7.2 ± 2.52
ΣMUFA	**20.74 ± 12.57**	**24.07 ± 4.72**	**26.89 ± 10.25**	**21.93 ± 9.73**
18:2n-6	1.13 ± 0.84	1.14 ± 0.72	0.8 ± 0.95	0.55 ± 0.91
20:4n-6 ARA	5.97 ± 1.9	5.68 ± 4.57	6.25 ± 2.49	5.78 ± 3.45
22:4n-6	0.69 ± 0.17	0.84 ± 0.79	0.76 ± 0.22	0.81 ± 0.56
22:5n-6	0.54 ± 0.11	0.48 ± 0.28	0.66 ± 0.37	0.7 ± 0.4
Σn-6 PUFA	9.26 ± 1.64	8.88 ± 5.74	9.64 ± 2.98	8.58 ± 4.98
18:3n-3	0.74 ± 0.94	0.43 ± 0.45	0.08 ± 0.13	0.11 ± 0.14
18:4n-3	0.75 ± 0.39^{ab}	1.15 ± 1.13^{a}	0.23 ± 0.41^{ab}	0.08 ± 0.2^{b}
20:4n-3	0.72 ± 0.56	0.57 ± 0.32	0.21 ± 0.2	0.29 ± 0.44
20:5n-3 EPA	8.64 ± 1.62^{a}	6.51 ± 3.7^{a}	2.25 ± 1.08^{b}	2.74 ± 1.73^{b}
22:5n-3	6.95 ± 2.22^{a}	4.96 ± 2.64^{ab}	2.97 ± 1.13^{b}	4.13 ± 2.17^{ab}
22:6n-3 DHA	17.09 ± 7.52^{a}	6.16 ± 1.99^{b}	8.75 ± 4.91^{ab}	12.86 ± 7.94^{ab}
Σn-3 PUFA	35.35 ± 7.85^{a}	20.07 ± 8.08^{b}	14.49 ± 6.52^{b}	20.31 ± 11.34^{ab}
ΣPUFA	**44.6 ± 8.64^{a}**	**28.95 ± 12.29^{ab}**	**24.13 ± 9.26^{b}**	**28.89 ± 15.45^{ab}**
DHA/EPA	1.9 ± 0.72^{a}	1.82 ± 2.05^{a}	4.33 ± 1.8^{b}	4.8 ± 0.95^{b}
ARA/EPA	0.69 ± 0.16^{a}	1.26 ± 1.39^{ab}	2.57 ± 0.89^{b}	2.35 ± 1.42^{b}
Total FA (mg g^{-1} lipids)	540.09 ± 28.94	691.89 ± 106.26	663.89 ± 75	607.08 ± 53.06

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Table 5. Estimation of total fatty acid composition (mg) of the entire organ / tissue for ΣSFA, ΣMUFA, Σn-6 PUFA, ARA, Σn-3 PUFA, EPA, DHA and ΣPUFA in flathead grey mullet females ovary at different maturation stages (n = 6 – 7 females per stage). Values are mean ± SEM. Values with different superscripts within rows indicate significant differences in total fatty acids (g) between different maturation stages.

	Estimation of total fatty acids (g) in the entire ovary			
ΣSFA	0.025 ± 0.004[a]	0.750 ± 0.253[a]	6.388 ± 1.486[b]	0.148 ± 0.046[a]
ΣMUFA	0.010 ± 0.002[a]	1.165 ± 0.392[a]	14.477 ± 3.071[b]	0.390 ± 0.268[a]
20:4n-6 ARA	0.010 ± 0.002[a]	0.159 ± 0.047[a]	1.496 ± 0.309[b]	0.082 ± 0.029[a]
Σn-6 PUFA	0.013 ± 0.003[a]	0.382 ± 0.113[a]	3.783 ± 0.777[b]	0.173 ± 0.083[a]
20:5n-3 EPA	0.010 ± 0.005[a]	0.286 ± 0.106[a]	1.374 ± 0.351[b]	0.042 ± 0.022[a]
22:6n-3 DHA	0.015 ± 0.009[a]	0.257 ± 0.095[a]	2.496 ± 0.653[b]	0.127 ± 0.050[a]
Σn-3 PUFA	0.033 ± 0.017[a]	0.858 ± 0.301[a]	5.894 ± 1.413[b]	0.263 ± 0.115[a]
ΣPUFA	0.046 ± 0.024[a]	1.240 ± 0.396[a]	9.677 ± 2.169[b]	0.437 ± 0.198[a]

Table 6. Fatty acid composition (% of total fatty acids) in flathead grey mullet female ovary at different maturation stages (n = 6 – 7 females per stage). Values are mean ± SD. Fatty acids with < 0.5 % are excluded. Values with different superscripts within rows indicate significant differences in fatty acid % between different maturation stages.

Fatty acid	Previtellogenesis	Early-vitellogenesis	Late-vitellogenesis	Post-spawning
14:0	0.49 ± 0.07^{a}	0.94 ± 0.19^{b}	0.52 ± 0.15^{a}	0.31 ± 0.11^{c}
15:0	0.63 ± 0.3	1.91 ± 1.97	2.5 ± 1.61	1.99 ± 1.05
16:0	19.14 ± 1.98^{a}	13.07 ± 2.09^{b}	8.65 ± 0.58^{c}	9.28 ± 4.22^{c}
18:0	9 ± 1.28^{a}	5.39 ± 1.77^{b}	6.33 ± 2.4^{ab}	8.39 ± 3.05^{ab}
ΣSFA	$\mathbf{29.32 \pm 1.85^{a}}$	$\mathbf{21.31 \pm 3.32^{b}}$	$\mathbf{18 \pm 3.74^{b}}$	$\mathbf{19.98 \pm 7.61^{b}}$
16:1	0.0 ± 0.0^{a}	11.93 ± 2.93^{b}	8.7 ± 4.11^{c}	3.96 ± 2.41^{d}
17:1	2.94 ± 0.5^{a}	11.34 ± 3.8^{bc}	14.65 ± 5.99^{c}	7.48 ± 7.79^{ab}
18:1n-9	3.45 ± 0.41^{a}	5.9 ± 2.95^{ab}	8.14 ± 2.23^{b}	6.57 ± 2.6^{ab}
18:1n-7	4.69 ± 0.68^{a}	5.27 ± 0.8^{ab}	9.21 ± 1.4^{c}	6.86 ± 2.36^{b}
ΣMUFA	$\mathbf{11.43 \pm 0.72^{a}}$	$\mathbf{31.8 \pm 9.57^{b}}$	$\mathbf{39.08 \pm 9.37^{b}}$	$\mathbf{24.49 \pm 14.92^{ab}}$
18:2n-6	0.43 ± 0.19	1.8 ± 1.39	0.88 ± 1.25	0.72 ± 1.07
18:3n-6	0.98 ± 0.35	3.11 ± 0.63	3.86 ± 0.89	2.85 ± 1.87
20:4n-6 ARA	12.26 ± 2.28^{a}	4.8 ± 1.82^{b}	4.25 ± 0.59^{b}	10.35 ± 4.98^{a}
22:4n-6	1.21 ± 0.34^{abc}	0.82 ± 0.36^{ab}	0.76 ± 0.12^{b}	1.89 ± 0.95^{c}
22:5n-6	0.91 ± 0.34^{abc}	0.63 ± 0.22^{ab}	0.72 ± 0.23^{b}	1.37 ± 0.61^{c}
Σn-6 PUFA	15.78 ± 2.9^{ab}	11.16 ± 3.55^{bc}	10.46 ± 1.48^{c}	17.16 ± 4.9^{a}
18:3n-3	0.17 ± 0.18^{a}	0.69 ± 0.34^{b}	0.39 ± 0.4^{b}	0.35 ± 0.51^{b}
18:4n-3	0.31 ± 0.18^{a}	0.89 ± 0.26^{b}	0.61 ± 0.24^{bc}	0.43 ± 0.22^{ac}
20:4n-3	0.36 ± 0.1^{a}	0.76 ± 0.34^{b}	0.45 ± 0.22^{ab}	0.31 ± 0.27^{a}
20:5n-3 EPA	12.38 ± 1.61^{a}	8.06 ± 3.53^{b}	3.7 ± 1.63^{c}	4.04 ± 1.03^{c}
22:5n-3	7.28 ± 1.12^{a}	6.21 ± 2.31^{ab}	3.9 ± 0.73^{b}	7.79 ± 2.74^{a}
22:6n-3 DHA	17.33 ± 2.25^{a}	8.43 ± 4.27^{b}	6.81 ± 1.26^{b}	13.59 ± 4.75^{a}
Σn-3 PUFA	38 ± 3.16^{a}	25.36 ± 8.47^{b}	15.98 ± 2.72^{c}	26.6 ± 6^{b}
ΣPUFA	$\mathbf{53.78 \pm 1.45^{a}}$	$\mathbf{36.53 \pm 9.09^{b}}$	$\mathbf{26.44 \pm 3.62^{c}}$	$\mathbf{43.76 \pm 9.57^{b}}$
DHA/EPA	1.43 ± 0.33^{a}	1.41 ± 1.16^{a}	2.16 ± 0.9^{ab}	3.42 ± 1.05^{b}
ARA/EPA	1.02 ± 0.28^{a}	0.78 ± 0.55^{a}	1.32 ± 0.5^{a}	2.64 ± 1.31^{b}
Total FA (mg g^{-1} lipid)	452.63 ± 90.36	548.52 ± 90.49	589.84 ± 56.15	456.37 ± 107.09

4. Discussion

In the present study, all flathead grey mullet females caught in the western Mediterranean were considered to be reproductively mature adults since they were larger (from 40 to 59.5 cm SL, 50.5 to 71 cm TL) than the reported size of maturation, which ranges widely; from 23 cm SL to 41 cm SL [25]. The available data on the sexual maturity of flathead grey mullet in the Mediterranean are scarce; they mostly refer to wild fish sampled on the coasts of Tunisia and Algeria [30,31]. Regarding the histological evaluation of gonadal maturation, females presented group-synchronous ovary development, which is characteristic of flathead grey mullets described in the eastern Mediterranean [26] or other mugilids [5]. At least two clutches of oocytes could be distinguished in the maturing ovary; a quite synchronous clutch of larger oocytes and a more heterogeneous population of smaller oocytes from which the clutch was recruited. The most developed oocytes were most likely to be spawned at once during the examined breeding season, while the others will be spawned in future breeding seasons, as is typical of total spawners. Ovarian recrudescence in Ebro Delta canals and waters of the western Mediterranean was observed during mid-summer. Early August was the first time that samples presenting the initiation of oocyte development —oocytes containing yolk droplets and, thus, vitellogenesis — were obtained. Further gonadal development was well described by the increase in GSI values. Females at advanced vitellogenesis, observed from mid-August to mid-September, showed an increase from 4.2 ± 3.6 % to 15.5 ± 3.4 %. Worldwide GSI values for fully mature flathead grey mullet females are, once again, highly variable. The highest GSI obtained in this study was 22.6%, similar to the reported values in the Mediterranean Sea that were around 25% [31,32]. The lowest GSI value for a mature flathead grey mullet (5.32 %) was obtained in the waters of Korea [33], while the highest GSI, close to 40 %, was obtained in the Gulf of Mexico [34]. The presence of females

with flaccid ovaries, prominent blood vessels full of postovulatory follicles, atresia, and low GSI (1.8 ± 0.9 %) in mid-September and mid-October, indicated the post-spawning period. Although no fish was sampled in spawning conditions, the present findings suggest that the species' spawning season in the western Mediterranean may occur during September and October; however, a larger number of individuals must be examined to be conclusive. Flathead grey mullet populations appear to have earlier spawning periods from the eastern to western Mediterranean. For instance, flathead grey mullet breeds from June to August in Turkey, June to September in Egypt, and August to October in Greece and Tunisia [24]. The reason why no spawning fish was observed in the current study could be that captures were conducted in nearshore marine zones and adult fish had already migrated to offshore areas to spawn. The flathead grey mullet is a catadromous fish; lives in inshore waters, enters in lagoons and estuaries, and spawns in the open sea [24].

In terms of lipid accumulation, flathead grey mullet female showed different body compositions depending on the seasonal increase of GSI during vitellogenesis. During the various gonadal stages, the muscle had the lowest lipid content (3.85 - 4.92%), which was within the range of previous mullet flesh analysis. The Mediterranean data corresponds to samples collected on the Turkish coast with a lipid content of 2.1 ± 0.1%. Nonetheless, it has been reported that the lipid content of flathead grey mullet muscle varies greatly, with more than a 10-fold range of values, depending on the geographic location. Therefore, comparisons between samples from different regions are limited [35]. According to the current findings, the flathead grey mullet female made little use of muscle tissue as a lipid depot. On the contrary, higher lipid levels (18.46 - 22.62%) were found year-round in the liver. Although no clear trend of liver lipid utilization was observed during the reproduction period, HSI% increased during vitellogenesis,

suggesting that changes in liver lipid content were happening during gonadal development. It is possible that there were differences in the different lipid fractions — neutral, comprising triacylglycerols and wax esters, and polar, consisting of membrane glycolipids and phospholipids— that were not revealed in the total. In the white seabream (*Diplodus sargus*), for example, the ratios of lipid fractions (neutral lipids / polar lipids) varied during gonadal development according to the utilization of lipid reserves [36]. Hence, investigating the different lipid fractions may yield more conclusive results about lipid dynamics in the liver. Peritoneal fat contained a high amount of lipids and was depleted as vitellogenesis initiated. Given the high variability in the presence of peritoneal fat between individuals in pre-vitellogenesis (45% of females) and in early-vitellogenesis (17% of females), it was probably transitional fat that could be rapidly metabolized or transferred to other tissues. It could be used for reproductive purposes or to ensure the additional requirement of metabolic energy during the coldest months of the year. Other fish species, such as the gilthead seabream, also store lipids in peritoneal fat [37]. A different trend was observed in the gonads. During vitellogenesis, total lipid content in the gonads increased from 4.90% to 34.59%, as expected to contribute to egg reserves [9]. The reported lipid content concerning raw roe of flathead grey mullet is scarce and it ranges from 13.1 to 23.3%, being the lowest in samples from a lagoon in Turkey [35]. In the present study, the increase in total lipid percentage in ovaries combined with the increase in GSI, indicates a massive accumulation of lipids from previtellogenesis to late vitellogenesis. Characteristically, the total lipid content of the entire tissue increased approximately 133-fold.

The profile of the fatty acid composition of the different tissues varied significantly during gonadal development. While there was no discernible change in the fatty acid profile of the muscle, there was in the liver and ovaries. The saturated fatty acid composition of

muscle, liver, ovaries, and peritoneal fat was mostly represented by the palmitic acid 16:0, with similar previously reported values in fillet and raw roe [38]. During vitellogenic development, the liver percentage of ΣSFA, mainly of 16:0, increased, suggesting that the liver would serve as a lipid depot for energy metabolization. In the ovaries, the estimated total SFA content significantly increased in the entire tissue at late-vitellogenesis, even though the percentage of ΣSFA decreased. This decrease was probably due to the respective increase in ΣMUFA percentages. As stated before, the peritoneal fat was mainly composed of 16:0 and was depleted through vitellogenesis, thus, it could be hypothesized that the 16:0 was used during gonadal maturation. Indeed, Henderson *et al*. [39] reported that the 16:0 fatty acid is the main source of energy metabolism for breeders, especially during the egg production period.

Regarding the mono-unsaturated fatty acid composition, palmitoleic acid (16:1) was the most abundant in muscle, contributing to 35 - 47 % of the total MUFAs, contrary to most fish species which is the oleic acid. High levels of 16:1 in the flesh have also been reported by Sengör *et al*. [38], Argyropoulou *et al*. [40], and Özogul and Özogul [41] and are characteristic of freshwater fish [40,41] which is consistent with the habitat use of the flathead grey mullet [24]. While MUFAs constituted half of the saturated fatty acids in the ovaries at previtellogenesis, the percentage doubled during vitellogenesis. The continuous accumulation of MUFA during gonadal development (up to 39.08 ± 9.37 %) and the subsequent decline after spawning, demonstrated their importance on the formation of embryo reserves. Our reported levels agree with published data for flathead grey mullet raw roe in other locations (13 - 42 %) [35]. Among MUFAs, 16:1 (palmitoleic acid), 17:1 (heptadecenoic acid), 18:1n-9 (oleic acid), and 18:1n-7 were preferentially accumulated throughout vitellogenesis. The accumulation of oleic acid in fish gonads during gonadal development has been widely reported in other fish species [36]. The

predominance of 16:1 in flathead grey mullet eggs has been previously reported and attributed to the diet [38]. Peritoneal fat was also highly composed of 16:1, suggesting the possible transference of this fatty acid to the ovaries. One interesting feature of the MUFA was the noticeably high levels of 17:1 in late-vitellogenic ovaries. The presence of 17:1 heptadecenoic acid has been previously reported as a trace element in flathead grey mullet muscle (< 0.1 – 1.94%) [38,41] and raw caviar (< 0.1 %) [38]. It has been found in low levels (≃ 1 %) in muscle, liver, and gonads of the Pacific herring (*Cuplea harengus pallasi*) [42], but has not been observed in other omnivorous species such as the white sea bream (*Diplodus sargus*) [36] or the golden grey mullet (*Liza aurata*) [43]. In the present study, while the 17:1 was not observed in muscle, peritoneal fat, or liver, it showed up to 14.65 ± 5.99 % levels in ovaries during vitellogenesis with a notable increase from previtellogenesis. This finding suggests that the 17:1 accumulation primarily depends upon the fish diet, reflecting an active feeding behavior during reproductive development. The fatty acids with an odd number of carbons, such as 17:1, likely originate from bacteria [38,41,44]. Given that the flathead grey mullet has been observed to feed on the bacterial scum of *Anabaena spp* [45], the high presence of 17:1 may indicate the relevance of bacteria in the diet as a source of lipids. Thus, future studies should address dietary 17:1 to elucidate its importance in female flathead grey mullet gonad maturation.

In female fish, it has been assumed that polyunsaturated fatty acids PUFAs are involved in the physiological reproductive processes; they play an essential role in the development of gonads, the formation of gametes, and the formation of cell membrane structures or ion channels regulation [9]. The high percentage of ΣPUFA (53.78 ± 1.45%) found in the ovaries during previtellogenesis is indicative of the importance of these fatty acids for the development of gonads. Although a significant decrease in ΣPUFA percentages (down to

26.44 ± 3.62 %) was observed during ovarian development, it was mainly due to the respective remarkable increase in ΣMUFA percentages. In fact, the estimated total PUFA content in ovaries increased at late-vitellogenesis and, therefore, the decrease in ΣPUFA % still represents a substantial increase in the content of the entire tissue.

Polyunsaturated fatty acids DHA, EPA, and ARA are essential fatty acids in marine fish. Docosahexaenoic acid and EPA have a structural role in membrane phospholipids [46] and are a source of metabolic fuel for reproduction [39]. Eicosapentaenoic acid and ARA are precursors for eicosanoids, including prostaglandins, thromboxanes, and leukotrienes, which are involved in steroidogenesis, oocyte maturation, and ovulation [9]. In marine fish, ARA and EPA compete for the enzymes that regulate eicosanoid production [47]; however, ARA forms more biologically active prostaglandins than EPA [48]. Different levels of ARA and EPA have been shown to influence prostaglandin production in wild and cultured Senegalese sole [49,50]. Higher levels of DHA, ARA, and EPA were found in the current study compared with muscle and raw caviar samples from flathead grey mullet in Sengör *et al*. [38]. Docosahexaenoic acid and ARA levels in muscle samples were also greater than those reported by Özogul and Özogul [41]. The high percentages of DHA, EPA, and ARA found in previtellogenic gonads suggest that the presence of these fatty acids is required for the onset of gonad development. The degree of difficulty in obtaining essential ARA differs among species, habitat, and food sources. High levels of ARA were also found in other fish species exhibiting an omnivore feeding strategy [51] and in a low trophic demersal feeding flatfish [52]. The ARA/EPA ratio in the liver showed an increasing trend, which could indicate that EPA accumulation in the liver was lower than that of ARA during ovarian development. A higher DHA/EPA ratio (greater than 2-3) was obtained in the liver and gonads at late vitellogenesis, which could also indicate a lower accumulation of EPA relative to DHA. High ratios were also obtained

during the post-spawning period, as EPA reserves were depleted while DHA remained relatively conserved. Overall, although PUFA and essential fatty acids (EPA, DHA, and ARA) decreased as a percentage, they still appeared to represent a significant increase in total ovarian content. This suggests that flathead grey mullet female breeder diets should include a variety of lipid classes, including PUFA, specifically EPA, DHA, and ARA, to ensure gonadal maturation, as recommended for another mugilid, the *Liza aurata* [43]. Providing large amounts of essential fatty acids to female breeders without using marine fish ingredients would be a future research challenge.

5. Conclusions

In conclusion, this study found that gonadal recrudescence and maturation in the flathead grey mullet are associated with increases in gonadal size, lipid accumulation, and changes in fatty acid composition. Endogenous lipids from the muscle are not used for gonadal growth in adult flathead grey mullet females. Contrarily, liver lipid dynamics may play a role in lipid distribution during vitellogenesis. One of the principal sources of the accumulation of all lipid classes, especially of 17:1, in ovaries during gonadal development appeared to be the diet. The importance of 17:1, however, remains to be elucidated. Depletion of peritoneal fat might also lead to lipid accumulation in the ovaries but it is challenging to determine since there is a high variability in its presence between individuals. Flathead grey mullet females require PUFA, primarily DHA, EPA, and ARA, for egg development, as well as MUFA, primarily 16:1, for the formation of embryo reserves. These findings will aid in the future development of broodstock-specific diets for the adequate ovarian development in this species.

Data availability

The data used to support the findings of this study are included within the article.

Conflict of interest

The authors declare no conflict of interest.

Authors' Contributions

Sandra Ramos-Júdez: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. **Alicia Estévez:** Formal analysis, Investigation, Validation, Writing - review & editing. **Wendy Ángela González-López:** Investigation, Writing - review & editing. **Neil Duncan:** Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing - review & editing.

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Figure 1. Macroscopic appearance of flathead grey mullet (*Mugil cephalus*) ovaries at different stages of maturity; (A) previtellogenic gonads, (B) gonads at early-vitellogenesis, (C) gonads at late-vitellogenesis, and (D) post-spawning ovaries. The scale bar in the figures indicates 5 cm.

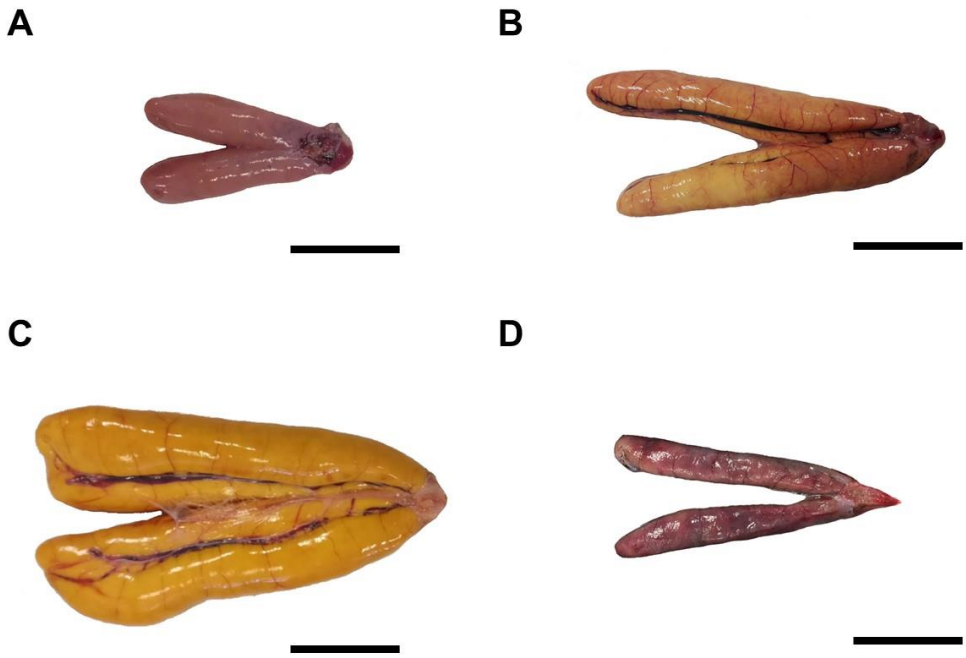
Figure 2. Light microscopic photograph of *Mugil cephalus* ovary at different maturity stages; (A) previtellogenic ovary rich in perinucleolar oocytes, (B) early-vitellogenic ovary with a dominant clutch of vitellogenic oocytes characterized by the inclusion of lipid droplets (arrowhead) and yolk granules (arrow) and a clutch of previtellogenic oocytes, (C) late-vitellogenic ovary with maximum size of oil droples and thickening of vitelline membrane in the most advanced clutch of oocytes, and (D) post-spawning ovary presenting post-ovulatory follicles (arrow), previtellogenic oocytes, and atresia (arrow head). Scale bar: 200 µm.

Figure 3. Variation in total lipid content (%) in the muscle, liver, ovaries and peritoneal fat of the flathead grey mullet (*Mugil cephalus*) across maturity stages of gonad development; previtellogenesis (n = 7) (PV), early-vitellogenesis (n = 6) (EV), late-vitellogenesis (n = 7) (LV), and post-spawn (n = 7) (PS). Data is presented as mean ± SD. Different letters show significant differences ($P < 0.05$) in each tissue along gonadal development.

Figure 4. Variation in the gonadosomatic index (GSI %) and hepatosomatic index (HPI %) respect to total lipids (%) in ovaries across maturity stages of gonad development; previtellogenesis (n = 7) (PV), early-vitellogenesis (n = 6) (EV), late-vitellogenesis (n =

725 7) (LV), and post-spawn (n = 7) (PS). Data is presented as mean ± SD. Different letters
726 show significant differences (P < 0.05) along gonadal development.
727
728 **Figure 5.** Total fatty acid content (%) of ΣSFA, ΣMUFA, oleic acid, Σn-6 PUFA, ARA,
729 Σn-3 PUFA, EPA and DHA in the muscle, liver and ovaries of the flathead grey mullet
730 (*Mugil cephalus*) female breeders at different stages of gonad development:
731 previtellogenesis (n = 7) (PV), early-vitellogenesis (n = 6) (EV), late-vitellogenesis (n =
732 7) (LV), and post-spawning (n = 7) (PS). Data is presented as mean ± SD. Different letters
733 show significant differences (P < 0.05) in each fatty acid percentage through gonadal
734 development.

735 **Figure 1.**



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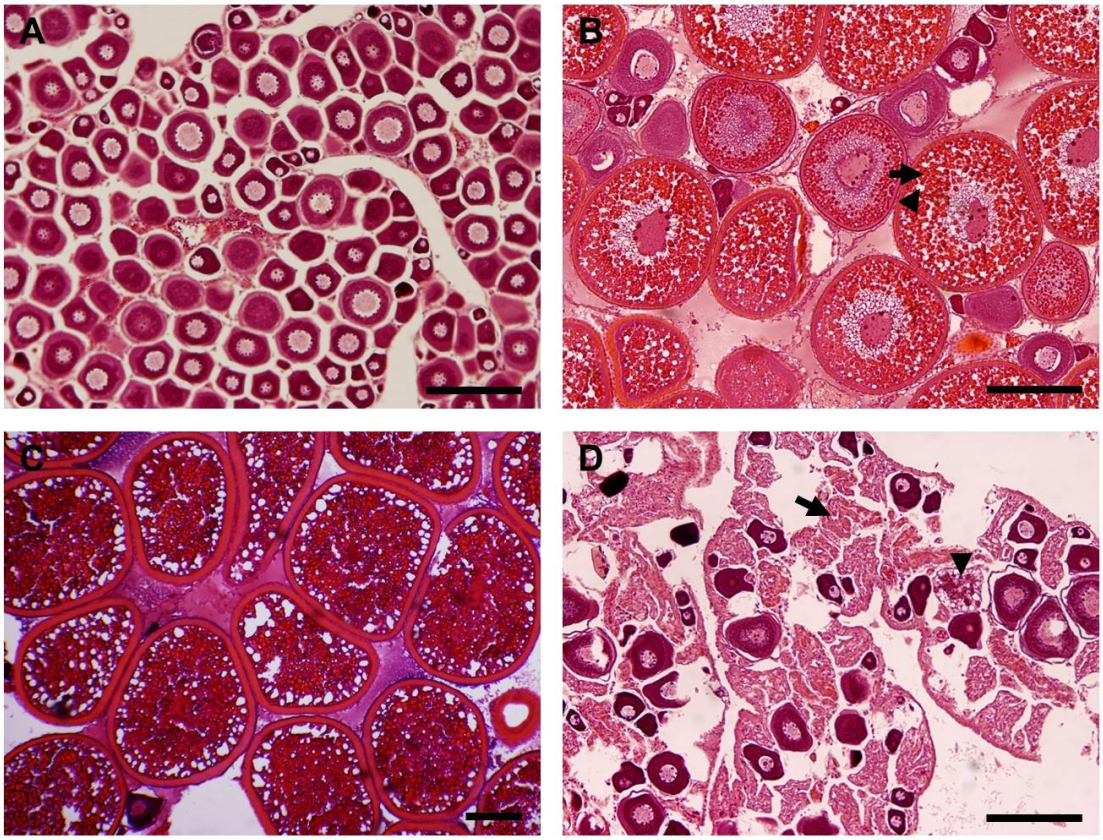
737 **Figure 2.**



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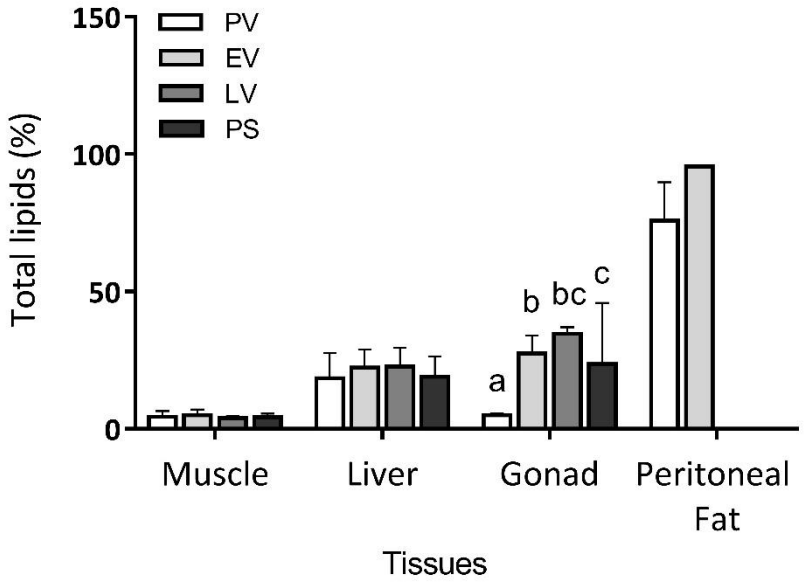
741 **Figure 3.**



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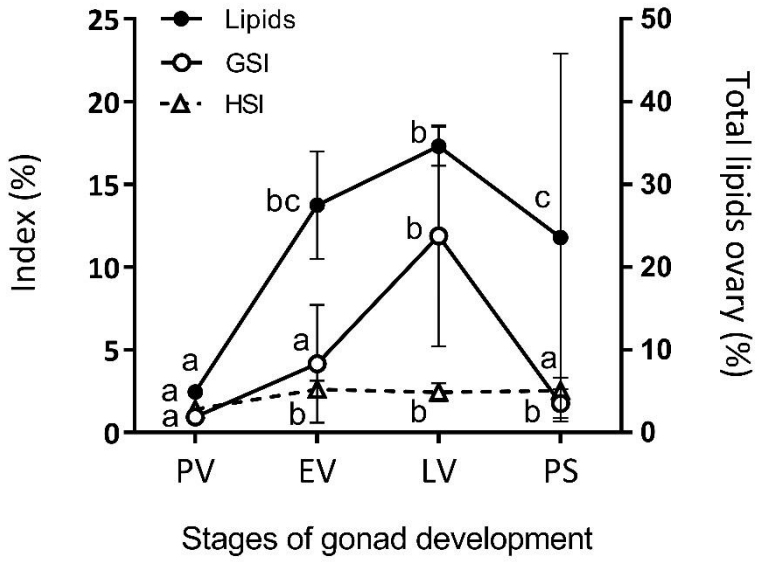
744 **Figure 4.**



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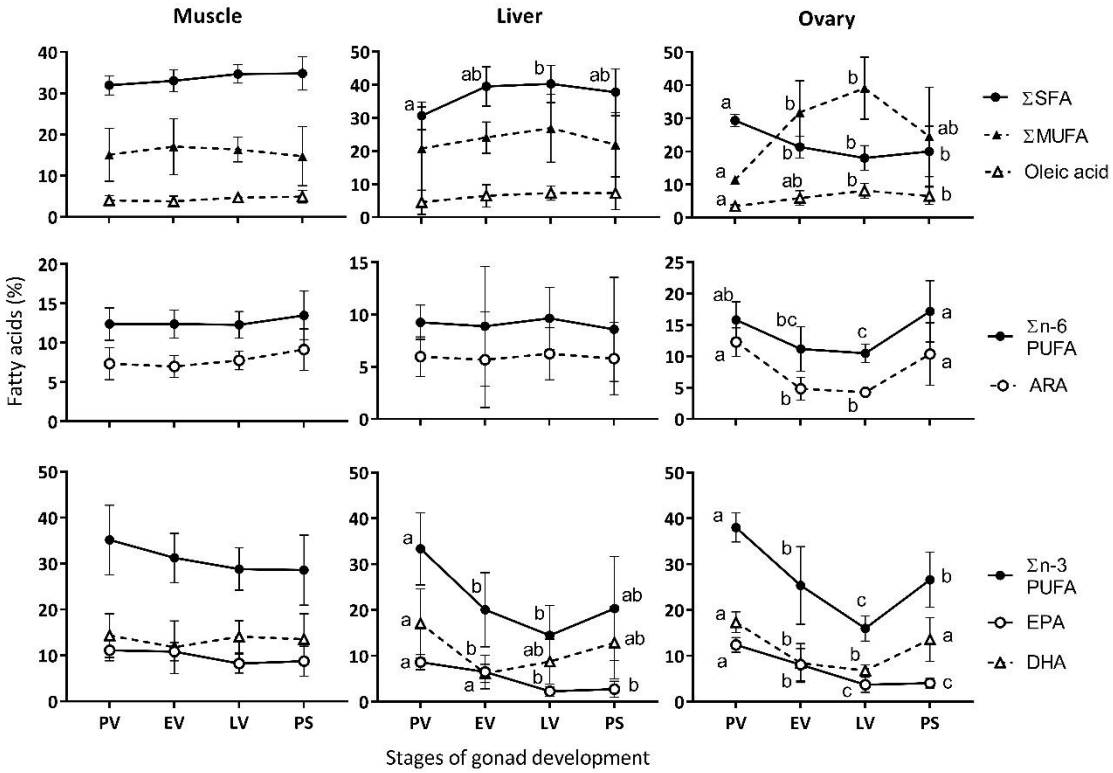
746 **Figure 5.**



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Highlights:

- Lipid and fatty acid dynamics in tissues of *Mugil cephalus*, an omnivorous teleost.
- Diet is one of the principal sources of lipids during ovarian growth.
- Novel identification of a high content of the 17:1 in teleost vitellogenic ovaries.
- Requirements of PUFAs for gonadal growth are such as for piscivorous species.