
Changes in lipid and fatty acid contents of gonad during the reproductive cycle of the Mediterranean swordfish *Xiphias gladius*

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Abstract :

Swordfish *Xiphias gladius* is a large pelagic fish distributed worldwide and exploited for human consumption, however there is limited knowledge about its reproductive biology, especially regarding lipid dynamic in gonads. In teleost fish, reproductive success and offspring survival are associated to lipid availability for gamete synthesis. This study investigated the lipid composition, including lipid classes and fatty acids (FA) of cell membrane and reserve lipids (i.e., polar and neutral lipids, respectively; PL and NL), along female and male gonad development of a swordfish population from waters surrounding Corsica Island in the Mediterranean Sea. Overall, swordfish gonads contained <2% wet weight of total lipids, with testes and ovaries having similar fat content. Lipid classes and FA concentrations remained unchanged during testes maturation. However, concentrations of phosphatidyl choline (PL), triacylglycerol (NL), and some FA (16:0, 18:1n-9, and 22:6n-3) followed an “inverted U-shaped” relationship with the ovarian maturation. In both PL and NL, 22:6n-3 was the main polyunsaturated FA (>20% of total FA), while 20:5n-3 and 20:4n-6 were minor (3–6% of total FA) and varied little with maturation. 22:6n-3 and 18:1n-9 were selectively allocated to the ovarian maturation (increased in concentration and in proportion with maturation) until spawning. Finally, swordfish gonads might represent a good food source for humans given that 150 g of swordfish ovaries can cover the daily requirements in omega-3 for humans, but research on pollutants should also be conducted to evaluate their implications on the reproduction output of this species, and on the safety of swordfish gonads for human consumption.

Highlights

▶ Swordfish gonads contain <2% wet weight of total lipids. ▶ Triacylglycerol and phosphatidyl choline are two main lipid classes of swordfish gonad. ▶ Concentrations of the main lipid classes and FA increase in ovary until spawning. ▶ 22:6n-3 and 18:1n-9 are selectively allocated to the ovarian maturation until spawning. ▶ Swordfish gonad represents a valuable food source of omega-3 for humans.

Keywords : ovaries, testis, maturity, omega-3, essentials fatty acids, large pelagic fish, human food source

46 1. Introduction

47 Swordfish *Xiphias gladius* is a large pelagic fish widely distributed in tropical and temperate
48 open waters, including the Mediterranean Sea (Palko et al., 1981). This species has a high
49 commercial value, and is fished mainly by longline and drifting gillnet. According to the
50 International Commission for the Conservation of Atlantic Tunas (ICCAT) who prepares the
51 stock assessment of swordfish in the Atlantic Ocean and adjacent seas, the Mediterranean
52 swordfish has been overfished for decades (ICCAT/SCRS, 2020), requiring the ICCAT
53 contracting parties to adopt a recovery plan for this stock. For the implementation of the
54 recovery plan, it is necessary to have fundamental knowledge on biological traits of the
55 Mediterranean stock of this species, including its reproductive biology (Murua and Saborido-
56 Rey, 2003; Neilson et al., 2013). Among the main information available to date, it can be
57 emphasized that the Mediterranean swordfish spawn during boreal summer (June to August)
58 with a peak of spawning occurring from late June to July; the size at first maturity (L_{50}) for
59 female is between 130 cm and 160 cm in lower-jaw-fork length (LJFL), and the batch fecundity
60 is high (ca. 6 millions oocytes) with spawning occurring in multiple batches (Arocha, 2007,
61 2002; Gomez Vives et al., 2005; Marisaldi et al., 2020). During the reproductive season,
62 swordfish concentrates into spawning grounds, which in the Mediterranean Sea are located in
63 the Levantine basin (east side), and in the Tyrrhenian and Ligurian Seas and around Balearic
64 islands (west side) (Di Natale et al., 2002; Garibaldi and Tserpes, 2020; Tserpes et al., 2008).
65 In teleost fish, reproduction requires high energy, mainly in the form of lipids, which is used
66 for the actual reproductive processes, the formation of gamete membranes and the constitution
67 of yolk reserve (Lubzens et al., 2017; Tocher, 2003). Reproductive success and offspring
68 survival are associated to lipid quality and availability for gamete synthesis (Marshall et al.,
69 1999; Rainuzzo et al., 1997). Lipid quality is a central element because lipids have vast
70 physiological functions: they act as structural component of (i) cell membranes and signalling
71 pathways, mainly in the form of phospholipids (which are polar lipids; PL), cholesterol, and
72 essential fatty acids, and (ii) energy storage, mainly in the form of triacylglycerol and sterol
73 esters (which are neutral lipids; NL). Essential fatty acids are long chain polyunsaturated fatty
74 acids (LC-PUFA) necessary for the correct organism function, but that consumers cannot
75 synthesize in sufficient quantity to meet their health requirements and that must thus be acquired
76 by their diet. Among LC-PUFA, optimal concentrations and proportions (ratios) in gonads of
77 20:5n-3 and 22:6n-3 (n-3 LC-PUFA family), and 20:4n-6 (n-6 LC-PUFA family), have been
78 shown to promote fecundity, egg hatching and viability, and larvae survival (Koven et al., 2018;
79 Lubzens et al., 2017; Rainuzzo et al., 1997). Furthermore, 22:6n-3 is especially required for

80 neural membrane formation in brain and eyes (Koven et al., 2018; Morais et al., 2011), while
81 20:5n-3 and 20:4n-6 mediate the inflammatory response and reproduction processes through
82 the production of eicosanoids and prostanoids (Chapkin et al., 2009; Xu et al., 2022). Contents
83 of n-3 and n-6 LC-PUFA are commonly studied relative to each other (ratios) to inform on
84 physiological state, given that eicosanoids from 20:5n-3 and 20:4n-6 have antagonist functions
85 and compete for their biosynthesis pathways, and that 20:5n-3 and 20:4n-6 can compete for
86 their incorporation into tissues, especially into phospholipids (Atalah et al., 2011; Sissener et
87 al., 2020).

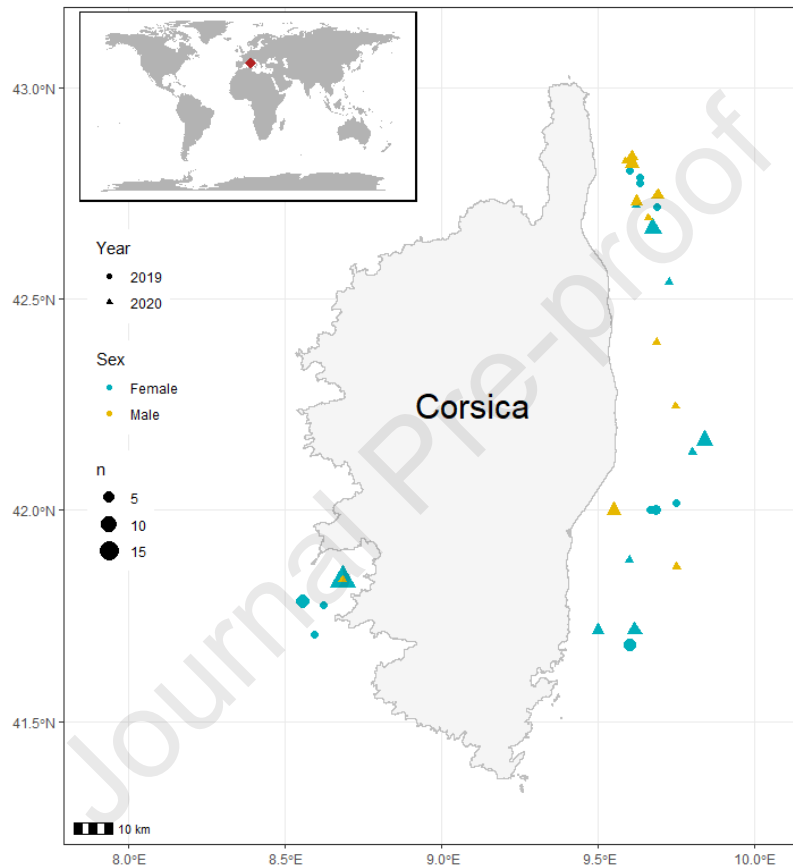
88 As most pelagic fish, the Mediterranean swordfish is rich in 22:6n-3, however its composition
89 varies significantly among individuals ($20 \pm 9\%$ of total FA in white muscle, Spain, $n=5$;
90 Cardona et al., 2015) and among tissues (28%, 18% and 15% of total FA in white muscle, brain,
91 and gonads, respectively, Tunisia, $n=6$; Ben Smida et al., 2009). In large pelagic fish, such
92 variabilities might be related to dietary changes (e.g., Cardona et al., 2015; Sardenne et al.,
93 2016; Young et al., 2010), seasonal migration (e.g., Goñi and Arrizabalaga, 2010; Lazo-
94 Andrade et al., 2021), and physiological changes, especially in relation to reproduction (e.g.,
95 Dhurmeea et al., 2018; Rangel et al., 2021). Lipid and FA requirements for swordfish
96 reproduction are however not documented, and energy allocation to reproduction is difficult to
97 measure in this species due to its indeterminate fecundity (i.e., the potential annual fecundity
98 of females is not fixed prior to the onset of spawning) (Arocha, 2002).

99 This study aims to contribute to the understanding of lipid requirement for swordfish
100 reproduction. To that purpose, we investigate variation in lipid composition in reproductive
101 tissues of females and males swordfish from the Mediterranean Sea. Specifically, we
102 investigated changes in lipid classes and FA content of both PL and NL from different
103 reproductive stages of ovaries and testis during the reproductive period of swordfish from
104 Corsica Island (west Mediterranean Sea). Finally, we also discuss the potential use of swordfish
105 gonads as a food source for human consumption.

106 2. Material & methods

107 2.1. Fish and gonad sampling

108 Seventy-eight fish were collected by longliners during the reproductive period of swordfish, in
 109 the boreal summers (June to September) of 2019 and 2020, near Corsica Island in the north-
 110 western Mediterranean Sea (Fig. 1). On-board, fishermen measured the fishes in LJFL to the
 111 nearest cm before gutting, and stored the whole gonads in ice. At landing, gonads were
 112 transhipped to the laboratory and weighted to the nearest gram (weight of the two gonads).



113
 114 **Fig. 1.** Sampling location (coordinates at the end of hauling) where swordfish (n=48 females
 115 and 30 males) were collected near the Corsica Island, Mediterranean Sea in 2019 and 2020.

116

117 2.2. Determination of swordfish reproductive stages

118 Reproductive stages were based on gonad examination and were determined on microscopic
 119 basis (histology) for ovaries, and on macroscopic basis (visual) for testes. Six reproductive
 120 stages were identified for females: immature (stage 1), developing (2a and 2b), spawning
 121 capable (3), spawning (4), regressing (or post-spawning) (5) and regenerating (6), according to
 122 the histological criteria of the 2021 ICCAT workshop on swordfish biology, based on Farley et
 123 al. (2016, 2013). Briefly, two subsamples (2 cm³) were taken in the central part of ovary and

124 were fixed in a 4% formaldehyde solution (10% formalin) and then preserved in 70% ethanol
125 for later histological analysis. Subsamples were dehydrated through a series of increasing
126 concentration of ethanol, cleared with NeoClear (i.e. substitute of xylene/toluene) and
127 embedded in paraffin. Histological section of 7 μm thickness were cut with a microtome from
128 the paraffin block, stained and examined under a microscope. Four reproductive stages were
129 identified for males: immature and developing (1), spawning capable (2), spawning (3) and
130 regressing (or post-spawning) (4), following the macroscopic criteria of ICCAT, based on
131 Poisson and Fauvel (2009).

132

133 2.3. Lipids analysis

134 2.3.1. Lipid extraction

135 Gonads sub-samples were freeze-dried for 72 hr and grounded to a homogeneous powder with
136 a ball mill. Moisture (i.e., water content) was measured as the difference between wet and dry
137 weights of samples (before/after freeze-drying), and expressed as percentage of wet weight.

138 Lipids were extracted from ca. 70 mg of dry powder with 6 mL of solvent mixture
139 (CHCl_3 :MeOH, 2:1, v:v) directly added into glass vials (Sardenne et al., 2019). Extracts were
140 flushed with nitrogen gas, vortexed and sonicated for 15 min to insure complete lipid extraction.

141

142 2.3.2. Lipid class analysis

143 Lipids classes were separated and quantified by High Precision Thin-Layer Chromatography
144 (HPTLC). HPTLC glass plates coated with silica were cleaned and then activated by heating
145 them at 120°C for 30 min. Lipid extracts and a mixture of external standards of known
146 concentrations were spotted on the plates with an automatic TLC sampler ATS4 (CAMAG).

147 Lipid classes were separated by immersion of the plates in solvent mixtures of different
148 polarities, which allows lipid migrations on the plates. First the plate was immersed in a solution
149 of methyl acetate:isopropanol: CH_2Cl_2 :MeOH:KCl 0.25% (10:10:10:4:3.6; v/v), allowing PL
150 separation. Subsequently the plates were immersed first in a solution of hexane:diethyl
151 ether:acetic acid (20:5:0.5; v/v) and then in a solution of hexane:diethyl ether (97:3; v/v), for
152 NL separation. Lastly the plates were immersed in a solution of CuSO_4 3% and H_3PO_4 8% (w/v
153 in distilled water) and then heated at 180°C for 30 min, to allow for final lipid class revelation.

154 The plates were read using a scanner densitometer set at 370 nm (TLC Scanner 4, CAMAG).

155 Lipid classes were identified and quantified by comparing retention time and band intensity of
156 each lipid class against these of known external standards using VisionCATS software (v2.4,
157 CAMAG). Mean analytical variability for lipid class quantification was about 15%. Five classes

158 of NL and six classes of PL were quantified: ALC=Alcohols, FFA=Free fatty acids, FS=Free
159 sterols, SE=Sterol- and wax- esters, and TAG=Triacylglycerol for NL; LPC=Lyo-
160 phosphatidyl choline, PC=Phosphatidyl-choline, SPG=Sphingomyelin, PS= Phosphatidyl-
161 serine PI=Phosphatidyl-inositol, and CL/PE=Cardiolipin and phosphatidyl-ethanolamine for
162 PL. Total lipid concentration was computed as the sum of all lipid classes. Concentrations were
163 expressed in mg.g^{-1} of wet weight.

164

165 2.3.3. Fatty acid analysis

166 2.3.3.1. Separation of neutral and polar lipids and trans-methylation

167 An aliquot of lipid extract (1 mL) was resuspended into $\text{CHCl}_3:\text{MeOH}$ (98:2; v/v). NL and PL
168 were then separated by adsorption chromatography at low pressure on a silica gel micro-column
169 (previously heated at 450 °C and deactivated with 6% deionized water (w/w)). NL were eluted
170 with 10 mL of $\text{CHCl}_3:\text{MeOH}$ (98:2, v/v), and PL with 20 mL of MeOH. NL and PL fractions
171 were dried under vacuum with an evaporator (Genevac). Once dry, 2.3 μg of an internal
172 standard was added (free fatty acid 23:0, 20 μL at 0.115 g.L^{-1}). All samples were transesterified
173 with 800 μL of $\text{MeOH}:\text{H}_2\text{SO}_4$ (3.4%; v/v), and heated at 100°C for 10 min. The formed fatty
174 acid methyl ester (FAME) were recovered in hexane.

175

176 2.3.3.2. Purification of fatty acid methyl-esters derived from neutral lipids

177 High Performance Liquid Chromatography (HPLC) equipped with two columns (LiChrospher
178 Si 60 and LiChrospher 100 DIOL, both 5 μm) and a Dionex HPLC system (P680 pump AS-
179 100 auto sampler, UVD170U UV detector with deuterium lamp, Foxy fraction collector), was
180 used to separate FAME from other unwanted compounds (e.g., sterols and alcohols), which are
181 contained in the NL fraction. Details on the analytical methods (i.e., solvent proportions, flow-
182 rate) can be found in Marty et al. (1999). The purified FAME were recovered in new vials for
183 gas chromatography analysis.

184

185 2.3.3.3. Gas chromatography

186 FAME composition was analysed by gas chromatography coupled to a flame ionization detector
187 (GC-FID; Varian CP8400 gas chromatograph, Agilent). Samples (2 μL) were injected at 250°C
188 in splitless mode at an oven temperature of 60°C, with hydrogen as the carrier gas. The GC was
189 equipped with a ZBWAX column (30 m in length, 0.25 mm internal diameter, 0.25 μm film

190 thickness, Phenomenex). The oven temperature was raised to 150°C at 50 °C.min⁻¹, to 170 °C at
191 3.5 °C.min⁻¹, to 185 °C at 1.5 °C.min⁻¹, to 225 °C at 2.4 °C.min⁻¹ and then to 250 °C at
192 5.5 °C.min⁻¹. FAME were identified by comparing their retention time to those of external
193 commercial standard mixture (S37 FAME Mix, PUFA n°1, and PUFA n°3, Supelco) using the
194 software Galaxie 1.9.3.2 (Agilent). FAME peak area was converted into µg of FA based on the
195 peak area of the internal standard 23:0. Mean analytical variability for FA quantification was
196 8.1%. Concentrations were expressed in mg.g⁻¹ of wet weight, and proportions in % of FAs
197 from NL and PL.

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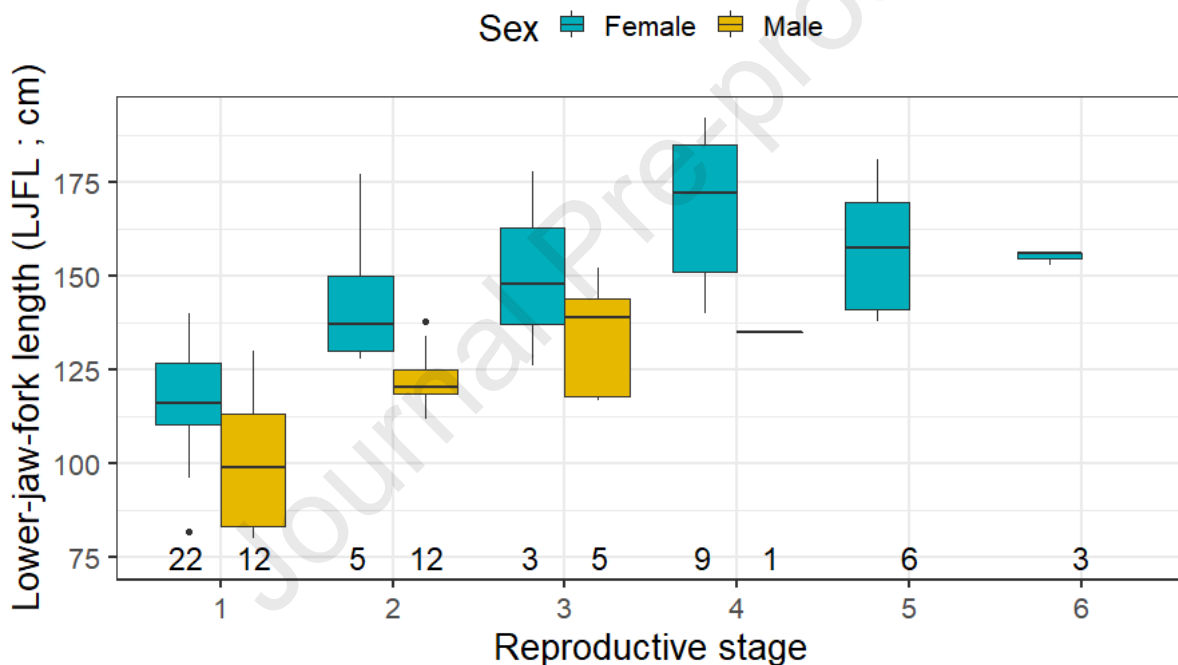
199 2.4. Data analysis

200 Total lipid allocated to gonads (TL, in gram) was computed as the gonads wet weight multiplied
201 by the concentration of the total lipids in wet weight. Eight outliers for lipid classes (3 males
202 and 5 females; total lipid content of 250-333 mg.g⁻¹) were removed from the dataset before
203 statistical analysis (technical issue during HPTLC analysis). To improve design balance,
204 females from maturity stage 2a (n=2) and 2b (n=3) were grouped under stage 2. However, the
205 design remained unbalanced (see section 3.1.) and homoscedasticity (Bartlett test) was not
206 reached for all lipid and FA concentrations. Because these statistical assumptions were not met,
207 non-parametric statistics were used to compare lipid and FA concentrations and ratios of
208 essential FAs between sexes and among maturity stages. Specifically, to investigate these
209 dissimilarities we used Kruskal-Wallis test (χ^2 statistic) to compare more than two groups,
210 Wilcoxon test (W statistic) to compare two groups, and Spearman correlation test (S statistic,
211 and ρ the correlation coefficient) to test for correlation between two variables. Principal
212 component analyses (PCA) were applied on untransformed FA percentage compositions to
213 visually examine FA profile. Data were analysed using R software 3.5.0 (R Development Core
214 Team et al., 2018).

215 3. Results

216 3.1. Size and year distributions by maturity stages

217 A higher number of female was caught in both years in comparison to male (48 vs. 30
 218 individual) (Table S1). For both sexes, individuals at a more advanced reproductive stage were
 219 the largest and immature individuals (stage 1) were the smallest (Fig. 2), resulting in co-
 220 variability between LJFL and reproductive stage ($\rho = 0.74$). The sampling design between
 221 reproductive stages and sampling year was also unbalanced, with most reproductive stages
 222 counting less than 5 individuals and most individuals at stages 1, 5 and 6 been caught in 2020
 223 (Fig. S1), making statistical comparisons between years impossible. But based on descriptive
 224 data, inter-annual differences in the total lipid content were not evident (Fig. S2).



225
 226 **Fig. 2.** Distribution of the lower jaw-fork length of swordfish *Xiphias gladius* caught from the
 227 Mediterranean Sea in 2019 and 2020 according to reproductive stages (from 1 to 6), with sex
 228 displayed by colour (female=blue, male=yellow). The box contains 50% of the data, the thick
 229 bar is the median value and the black dots are outliers. Numbers at the bottom of the panel
 230 indicate the number of individuals (n) for each group.

231

232 3.2. Content of water, total lipid, and lipid classes with reproductive stages

233 Average water content was higher in testes than in ovaries ($81 \pm 3\%$ vs $77 \pm 4\%$; $\chi^2=18.7$,
 234 $p<0.001$), and for the latter, it followed a “U-shaped” relationship with maturity stages, i.e.
 235 water content was the highest at the least (stages 1 and 2) and most advanced (stage 6)

236 reproductive stages (Table 1; $\chi^2=29.6$, $p<0.001$). Average total lipid concentration was $18.1 \pm$
 237 8.1 mg.g^{-1} , i.e. $1.8 \pm 0.8\%$ of gonad wet weight (min–max = $7.5\text{--}46.3 \text{ mg.g}^{-1}$; $0.8\text{--}4.6\%$), and
 238 it was similar in ovaries and testes (17.6 ± 9.6 vs $18.8 \pm 5.2 \text{ mg.g}^{-1}$; $\chi^2=3.2$, $p=0.07$), with a high
 239 inter individual variability (coefficient of variation = 45%) according to reproductive stage
 240 ($\chi^2=22.2$, $p<0.001$) (Fig. 3).

241 In detail, ovaries weight and TL varied with reproductive stage ($\chi^2= 37.5$, $p<0.001$ and $\chi^2=$
 242 31.4 , $p<0.001$, respectively) following an “inverted U-shaped” relationship (Table 1). Ovaries
 243 weight increased from stage 1 ($68 \pm 39 \text{ g}$) to stage 4 ($2888 \pm 2126 \text{ g}$), then decreased to stage 6
 244 ($353 \pm 77 \text{ g}$). An increase of weight and TL of testes was observed from stages 1 to 3 ($\chi^2= 21.0$,
 245 $p<0.001$ and $\chi^2= 21.1$, $p<0.001$, respectively), with the weight increasing from $12 \pm 6 \text{ g}$ to 107
 246 $\pm 56 \text{ g}$. The changes observed in the TL of the gonads (in grams) were associated to changes in
 247 both gonad weight and concentration of total lipids.

248

249 **Table 1.** Mean \pm standard deviation (SD) of gonad water content (in %), wet weight and total
 250 lipid (i.e., TL, gonads weight multiplied by the concentration of the total lipids in wet weight)
 251 expressed in grams, according to reproductive stage.

	Water content (%)		Gonad wet weight (g)		Gonads TL (g)	
	Ovaries	Testes	Ovaries	Testes	Ovaries	Testes
Stage 1	80.0 ± 1.6	80.0 ± 2.9	68 ± 39	12 ± 6	0.8 ± 0.4	0.2 ± 0.1
Stage 2	78.2 ± 2.6	81.6 ± 2.7	335 ± 177	47 ± 29	4.8 ± 3.5	0.9 ± 0.5
Stage 3	70.3 ± 5.5	83.1 ± 1.3	720 ± 411	107 ± 56	19.9 ± 14.8	1.9 ± 0.3
Stage 4	72.9 ± 4.6	82.2	2888 ± 2126	23	103.2 ± 107.4	0.3
Stage 5	75.5 ± 2.7	–	578 ± 161	–	13.2 ± 6.4	–
Stage 6	75.0 ± 2.7	–	353 ± 77	–	4.9 ± 1.6	–

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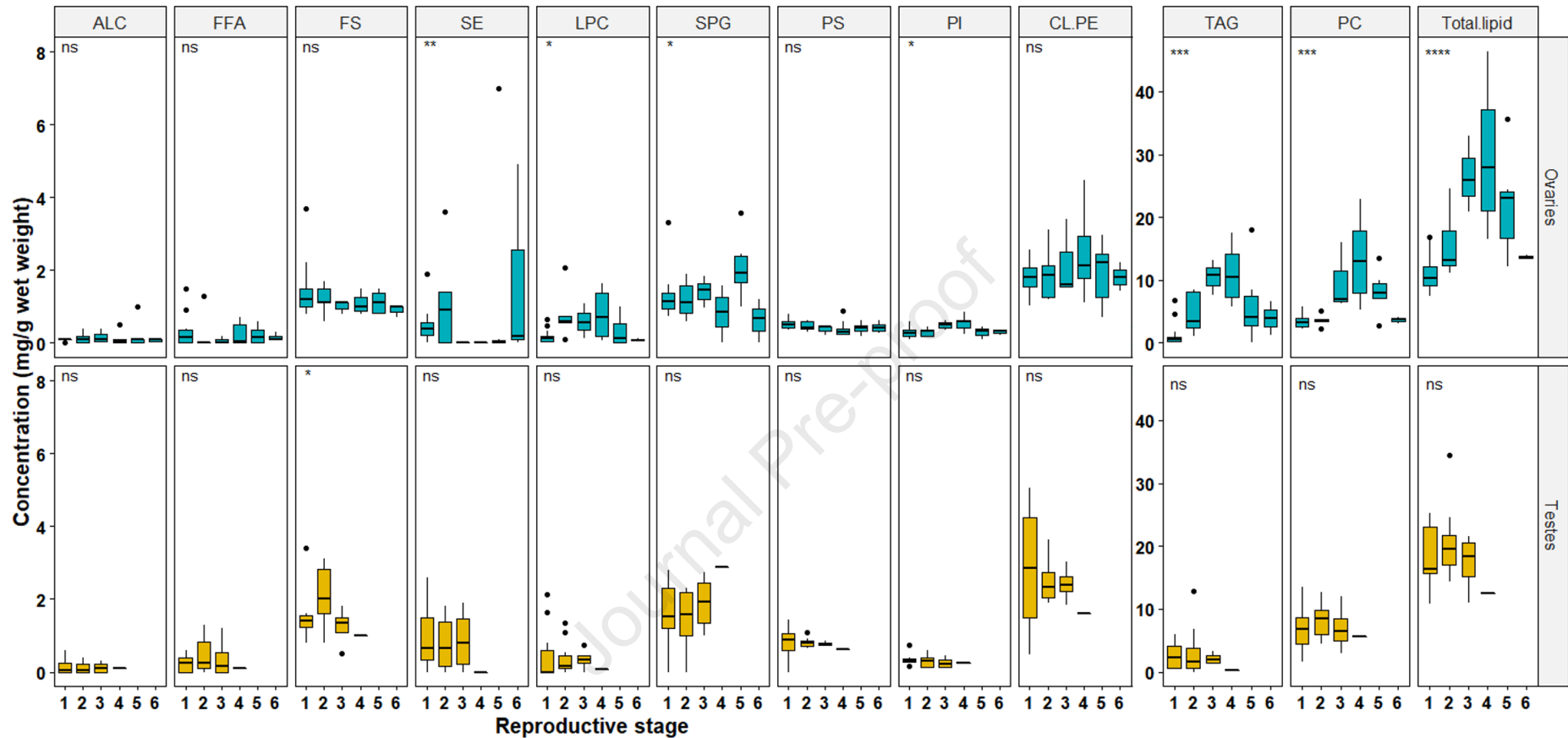
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254 Regardless of maturity stages and sex, the ratio between the sum of all NL or all PL lipid classes
 255 ranked from 0.1 to 1.4. This ratio was higher in ovaries than in testes (0.7 ± 0.4 vs 0.5 ± 0.4 ;
 256 $\chi^2= 4.9$, $p<0.05$), and tended to increase with reproductive stages in ovaries (Fig. S3).

257 Regardless of sexes and reproductive stages, PC, TAG and CL/PE were the three main lipid
 258 classes (Fig. 3), which averagely accounted for 35%, 18% and 13% of total lipids, respectively,
 259 followed by SE (5% of total lipids). However, some differences were observed between sexes
 260 and maturity stages: TAG, PC, and PI concentrations increased until stage 4 in ovaries (Fig. 3;
 261 top panels). TAG concentration increased more than 9-fold between stages 1 and 4, from $1.2 \pm$
 262 1.6 to $10.9 \pm 4.3 \text{ mg.g}^{-1}$, and then it decreased to $3.9 \pm 3.8 \text{ mg.g}^{-1}$ at stage 6. PC concentration
 263 tripled between stages 1 and 4 from 3.4 ± 1.0 to $13.2 \pm 6.9 \text{ mg.g}^{-1}$, and then it decreased to 3.6
 264 $\pm 0.5 \text{ mg.g}^{-1}$ at stage 6. Changes in PI concentrations were small but followed the same trend of

265 PC (from 0.3 ± 0.1 to 0.5 ± 0.2 mg.g⁻¹ between stages 1 and 4). In contrast, in testes lipid classes
266 changed little with reproductive stages, apart from a slightly higher concentration of FS at stage
267 2 as compared to the other stages (Fig. 3; bottom panels).

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Fig. 3. Distribution of lipid class concentrations in swordfish gonads (in mg.g⁻¹ wet weight), according to the reproductive stages (1 to 6) and fish sex (female/ovaries: top panels, male/testes: bottom panels). See Material & Methods for the acronyms definition of the eleven lipid classes. Symbols on the top left of each panel indicate concentration differences with reproductive stages according to Kruskal-Wallis tests: ns=not significant, *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

273 3.3. Changes in fatty acid composition with reproductive stages

274 Overall, FAs of swordfish gonads were mainly composed of 22:6n-3, 16:0, 18:1n-9, 18:0,
 275 20:4n-6, and 20:5n-3 in both NL and PL, and these six FA accounted for up to 90 % of neutral
 276 and polar FA (Tables 2 and S2). Regardless of sexes, FA profiles differed between NL and PL
 277 with PL containing more 20:4n-6 and 22:5n-6, and NL containing more 20:4n-3, 18:2n-6 and
 278 18:1n-9 (discrimination on PC1; Fig. 4). PL contained more SFA and PUFA than NL (W=1596,
 279 $p<0.001$ and W=1977, $p<0.01$, respectively), while similar concentrations of monounsaturated
 280 FA (MUFA) were observed between these fractions (W=2746, $p=0.70$) (Tables 2 and S2).
 281 Regardless of lipid fractions, some differences were also noted between sexes, with ovaries
 282 containing more PUFA than testes (2.4 ± 1.9 vs 1.6 ± 1.0 mg.g⁻¹; W=3298, $p<0.05$), especially
 283 n-6 PUFA (W=3454, $p<0.01$).

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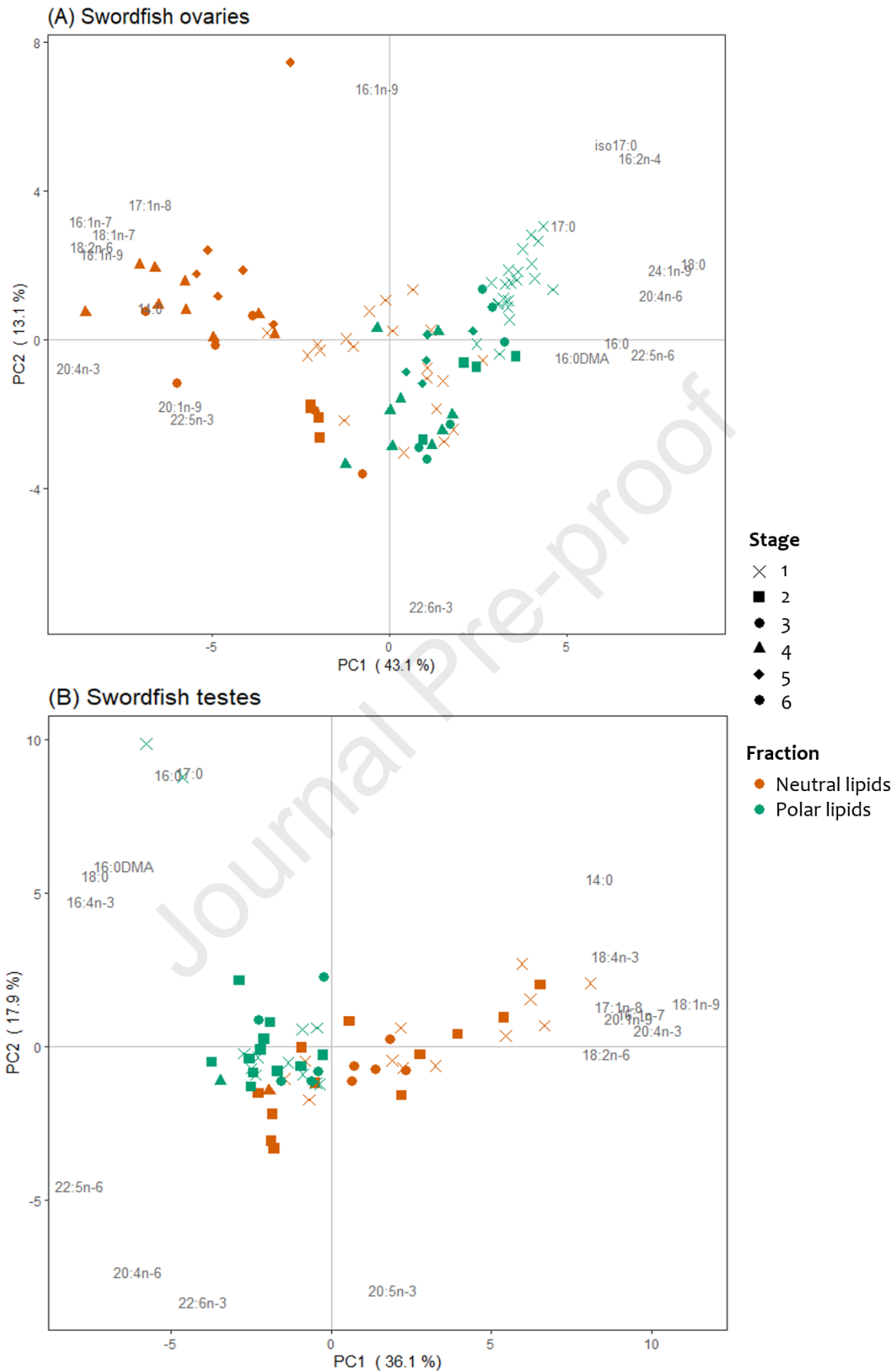
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286 **Table 2.** Mean \pm standard deviation (SD) of the ten fatty acids contributing the most to the total
 287 fatty acids, and the sum of all saturated, monounsaturated and polyunsaturated fatty acids in
 288 neutral lipids (NL) and polar lipids (PL) of swordfish ovaries and testes, expressed in mg.g⁻¹
 289 wet weight (see Table S2 for data expressed in %).

290

	Ovaries		Testes	
	NL	PL	NL	PL
<i>Saturated fatty acids (SFA)</i>				
16:0	1.0 \pm 1.0	1.1 \pm 0.6	0.6 \pm 0.6	1.0 \pm 0.2
18:0	0.3 \pm 0.3	0.4 \pm 0.2	0.3 \pm 0.2	0.4 \pm 0.1
Σ SFA	1.5 \pm 1.4	1.8 \pm 0.8	1.0 \pm 0.9	1.6 \pm 0.3
<i>Monounsaturated fatty acids (MUFA)</i>				
16:1n-7	0.2 \pm 0.3	0.1 \pm 0.0	0.1 \pm 0.1	0.1 \pm 0.0
18:1n-7	0.3 \pm 0.2	0.1 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.0
18:1n-9	2.1 \pm 2.4	0.6 \pm 0.3	0.8 \pm 0.9	0.8 \pm 0.2
24:1n-9	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0
Σ MUFA	2.8 \pm 3.2	1.0 \pm 0.4	1.2 \pm 1.2	1.2 \pm 0.3
<i>Polyunsaturated fatty acids (PUFA)</i>				
20:4n-6	0.2 \pm 0.1	0.3 \pm 0.1	0.1 \pm 0.1	0.2 \pm 0.1
20:5n-3	0.3 \pm 0.3	0.2 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.1
22:5n-3	0.2 \pm 0.2	0.1 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.0
22:6n-3	1.5 \pm 1.4	1.4 \pm 0.9	0.7 \pm 0.7	1.2 \pm 0.5
Σ PUFA	2.5 \pm 2.5	2.3 \pm 1.2	1.2 \pm 1.1	2.0 \pm 0.7

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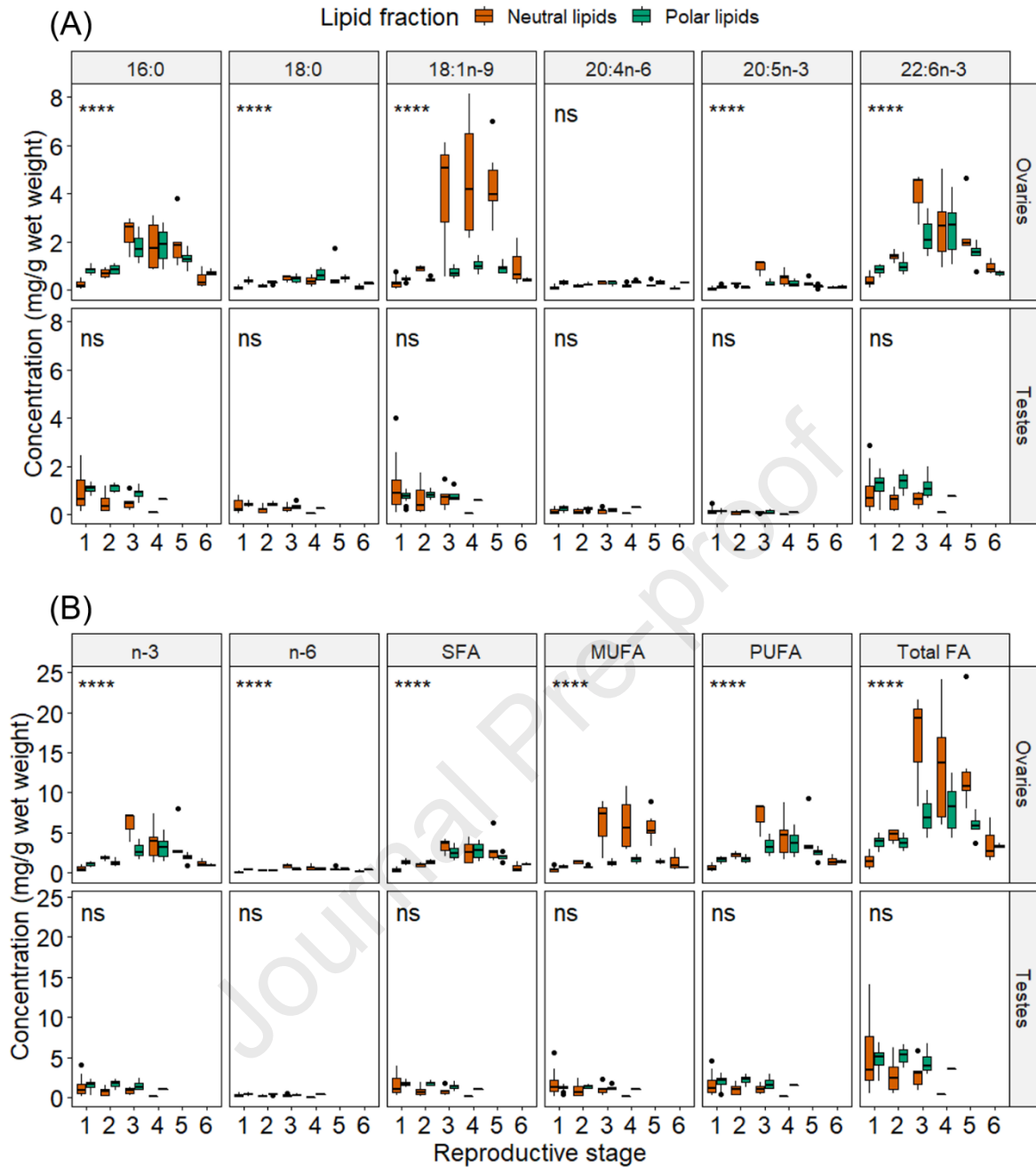


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293 **Fig. 4.** Principal component analyses (PCA) of FA percentage composition in neutral (red) and
 294 polar (green) lipids of (A) ovaries and (B) testes of the Mediterranean swordfish *Xiphias*
 295 *gladius*, according to the reproductive stages (1 to 6, displayed by dot shape).

296
297 FA profiles of NL and PL also differed with reproductive stages, especially in ovaries (Fig. 4).
298 Main changes in FA concentrations and proportions were observed during ovaries maturation
299 while no changes were observed during testes maturation (Fig. 5 and S3). In ovaries,
300 concentrations of 16:0, 20:5n-3 and 22:6n-3 significantly increased from stages 1 to 3/4 then
301 decreased until stage 6 in both NL and PL (i.e., “inverted U-shaped” relationships).
302 Concentration of 18:1n-9 increased from stages 1 to 4, in particular in NL (Fig. 5A; top panels).
303 In contrast, concentrations of 18:0 and 20:4n-6 remained stable throughout the gonad
304 maturation in both NL and PL ($0.2 \pm 0.1 \text{ mg.g}^{-1}$; Fig. 5A). Changes in the main FA families
305 followed that of their main FA, in particular changes in saturated FA paralleled changes in 16:0,
306 and changes in n-3 PUFA paralleled changes in 22:6n-3 (Fig. 5B).
307 For some FA, change in concentration with the ovarian maturity was directly related to the
308 change in total lipid concentration described in section 3.2 (e.g. concentration of 16:0 increase
309 between stages 1 and 4 as the total lipid concentration, without changing the proportion/relative
310 contribution of 16:0 to the FA profile; Fig 5 and Fig S3A). Conversely, for other FA, the
311 increase in concentration with the ovarian maturity was accompanied by an increase in their
312 proportions/relative contribution to the total FA. For instance, 18: 1n-9 increased in both
313 concentration and proportion of NL between stages 1 and 4; 22: 6n-3 increased in both
314 concentration and proportion of PL between stages 1 and 4 (Fig 5 and Fig S3A).

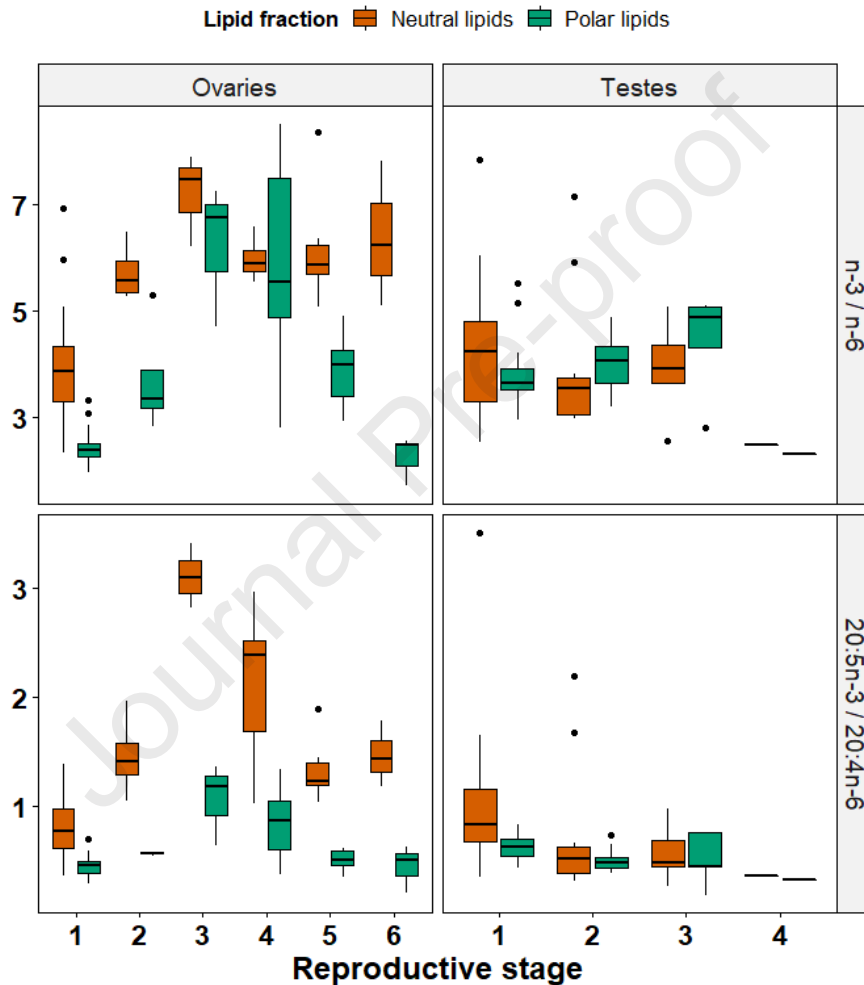
315



316
 317 **Fig. 5.** Concentrations of (A) the six main fatty acid (FA) and (B) FA families, in neutral (red)
 318 and polar (green) lipids of the gonads of the Mediterranean swordfish *Xiphias gladius* (in mg.g⁻¹
 319 of wet weight), according to the reproductive stages (1 to 6) of females (ovaries: top panels)
 320 and males (testes: bottom panels). Symbols on the top left of each panel indicate concentration
 321 differences with reproductive stage (regardless of the lipid fraction), according to Kruskal-
 322 Wallis tests: ns=not significant, *= p<0.05, **= p<0.01, ***= p<0.001, ****= p<0.0001. n-3 =
 323 omega-3 FA, n-6 = omega-6 FA, SFA = Saturated FA, MUFA = Monounsaturated FA, PUFA
 324 = Polyunsaturated FA.

325

326 Regarding FA ratios, n-3/n-6 ranked 1.7–8.5, 20:5n-3/20:4n-6 ranked 0.2–3.5, and 22:6n-
 327 3/20:5n-3 ranked 1.1–21.9. Regardless of sex, both n-3/n-6 and 20:5n-3/20:4n-6 ratios were
 328 higher in NL than in PL ($\chi^2=18.5$ and $\chi^2=34.3$, both $p<0.0001$) (Fig. 6). Main changes in FA
 329 ratios with reproductive stages concerned the n-3/n-6 and 20:5n-3/20:4n-6 ratios of ovaries,
 330 which followed an “inverted U-shaped” relationship (Fig. 6; left panels), while it did not
 331 significantly change in testes.
 332



333
 334 **Fig. 6.** Ratios of fatty acids (n-3/n-6 and 20:5n-3/20:4n-6; no unit) in neutral (red) and polar
 335 lipids (green) lipids according to reproductive stages of female (left panel) and male (right
 336 panel) Mediterranean swordfish. To note the scale used in each panel is different.

337 4. Discussion

338 Lipids are energetic reserve and structural components of any living organisms, and are
339 involved in reproductive processes. To contribute to an understanding of the reproductive
340 biology of Mediterranean swordfish, lipid and FA contents in gonads were studied during the
341 reproductive cycle of male and female swordfish from the Mediterranean Sea. While lipid
342 classes and FA concentrations remained unchanged during the reproductive cycle of males,
343 concentrations of PC, TAG, 16:0, 18:1n-9, and 22:6n-3 followed an “inverted U-shaped”
344 relationship with ovarian maturation. Their concentrations increased from immature (stage 1)
345 to spawning-capable stages (stages 3/4, corresponding to eggs emission) before decreasing
346 during the regressing and regenerating phases (i.e., stages 5/6). This suggest that these
347 compounds are of particular importance for swordfish eggs production. However, a note of
348 caution is warranted due to the low number of samples available for the analysis between
349 reproductive stages 2 to 6, and to the need to account also for the lipid composition of other
350 tissues (liver and muscle) at different seasons.

351

352 Lipid and fatty acid functions during gonad maturation of swordfish

353 During fish ovarian maturation, oocytes growth involves high accumulation of materials and
354 nutrients, including phospholipid-rich yolk proteins precursors ('vitellogenins') and energetic
355 reserve ('oil droplets') which are used to sustain the progeny (Lubzens et al., 2017; Sullivan
356 and Yilmaz, 2018). Average total lipids was 1.8% of ovaries wet weight in swordfish, with NL
357 (TAG + SE) reaching $36.8 \pm 7.3\%$ of total lipids in the spawning stages (stages 3/4). This result
358 suggests that swordfish spawns floating eggs with relatively large oil droplets. Similarly,
359 significant oil droplets are usually detected in swordfish in late stage vitellogenic oocytes by
360 imagery examination (Arocha, 2002; Carnevali et al., 2019; Ortiz-Delgado et al., 2008). Several
361 large pelagic fish have similar lipid content of ovaries as the one of this study (0.8-4.6% ww),
362 with an accumulation of phospholipids and TAG before spawning. These include the yellowfin
363 tuna *Thunnus albacares* (0.5-2.7% ww) (Zudaire et al., 2014), albacore tuna *Thunnus alalunga*
364 (0.8-5.2% ww) (Dhurmeea et al., 2018), skipjack tuna *Katsuwonus pelamis* (0.5-6.6% ww)
365 (Grande et al., 2016; Hiratsuka et al., 2004), and Pacific Bluefin tuna *Thunnus orientalis* (0.5-
366 10.1% ww) (Hiraoka et al., 2019). These reports and our results suggest that PC and TAG
367 deposition in ovaries is a general strategy used by large fish species of the Scombroidei sub-
368 order for the production of eggs, which is common in marine fish (Wiegand, 1996).

369 Allocation of phospholipids, especially of PC (35% of total lipids here), to maturing oocytes
370 might improve future embryo and larvae development. Diets rich in phospholipids, especially
371 PC, improve growth and survival rate while decreasing malformation of fish larvae (Cahu et
372 al., 2009; Reis et al., 2020; Tocher et al., 2008). Lipids such as TAG (reaching ca. 40% of total
373 lipids in ovaries of stage 3/4) are important for fish eggs as energetic reserve, and SE (5% of
374 total lipids in swordfish ovaries) are usually involved in yolk energy storage and probably to
375 egg buoyancy, due to their low specific gravity (Anderson et al., 1990; Lubzens et al., 2017).
376 Regarding swordfish testes, lipid class and FA concentrations remained constant throughout
377 the maturation process. Nevertheless, testes were as high in lipids as ovaries during all the
378 reproductive cycle (ca. 2% ww), especially in membrane lipids PC and CL/PE. However, the
379 macroscopic determination of maturity stages prevent a firm conclusion regarding lipid
380 requirements for male reproductive success. In comparison to Atlantic Bluefin tuna, swordfish
381 testes had higher PC and CL/PE proportions (23% and 16% of total lipids in swordfish,
382 respectively *versus* 10% of total lipids for both classes in Bluefin tuna; Sprague et al., 2012),
383 however the physiological reason for this remains still to be determined.

384 PUFA such as 22:6n-3, 20:5n-3, and 20:4n-6 are essential for the development of fish larvae,
385 and their proportions and ratios are species-specific (Sargent et al., 1999; Xu et al., 2022).
386 22:6n-3 was the main essential PUFA in swordfish gonads, as it is usually the case in other
387 large pelagic fishes (e.g. Dhurmeea et al., 2018; Hiraoka et al., 2019; Sardenne et al., 2017). Its
388 increasing concentration and proportion along the ovarian maturation highlighted the selective
389 allocation of 22:6n-3 to ovaries, especially to phospholipids, until the spawning stage (stage 4),
390 and the importance of 22:6n-3 for swordfish egg production. 22:6n-3 bounded to PE and PC is
391 indeed of high importance for correct development of fish larvae, as di-22:6n-3 phospholipids
392 are required for the retinal development, especially for predatory fish larvae (Wiegand, 1996).
393 This increased requirement of 22:6n-3 can be fulfilled by diet or by the remobilisation of lipid
394 reserves from other tissues, especially liver, as it was observed for the Pacific Bluefin tuna
395 (Hiraoka et al., 2019). 20:4n-6 and 20:5n-3 are PUFA used to regulate inflammatory responses
396 and reproductive processes through eicosanoids synthesis, and their low amounts (in
397 comparison to 22:6n-3) recorded in our study might be related to their conversion by elongation
398 into 22:4n-6 and 22:5n-3 (Scholefield and Schuller, 2014). Such regulation could be related to
399 the gonad maturation since the percentages of 20:4n-6 and 20:5n-3 decreased with ovary
400 maturation, a pattern that has been observed in several fish species (Xu et al., 2022, 2017).
401 However, this regulation remains to be further investigated since concentration changes with

402 maturity stages were observed for 22:6n-3 and at a lesser extent for 20:5n-3, but not for 20:4n-
403 6. The n-3/n-6 and the 20:5n-3/20:4n-6 ratios followed an “inverted U-shaped” relationship
404 with ovarian maturation. Overall, the n-3/n-6 ratio ranked 1.7–8.5, which is in close range with
405 values of tropical tunas (1.0–5.2, ovaries and testes; Sardenne et al., 2017) and albacore tuna
406 (1–6.5, ovaries only; Dhurmeea et al., 2018), and slightly lower than Pacific Bluefin tuna (9.3,
407 ovary only; Hiraoka et al., 2019), suggesting large pelagic fishes have similar and high n-3
408 needs for gonad maturation and eggs production. The high concentration of 18:1n-9, especially
409 in reserve lipids of mature ovaries (stages 3 to 5), could be related to the MUFA role in
410 metabolic energy production, as they are among the preferred substrates for β -oxidation
411 (Henderson and Sargent, 1985; Sidell et al., 1995). The increase in 18:1n-9 concentration and
412 proportion with the ovarian maturation suggest that this FA is selectively allocated to the
413 ovaries during maturation, and later consumed for energy supply during embryogenesis and
414 larval development.

415

416 **Maternal effect & energy allocation in swordfish**

417 Maternal effect is a non-genetic contribution of females to offspring fitness. It generally results
418 in the fact that larger and older females may produce more- and higher quality- offspring
419 (Barneche et al., 2018; Green, 2008), which can influence stock recruitment and stability (“big
420 old fat fecund female fish” concept; Hixon et al., 2014). For fisheries resource management,
421 female demography and inter-female differences deserve consideration in developing
422 harvesting strategies (Barneche et al., 2018; Green, 2008). A maternal effect was suggested for
423 swordfish from the Indian Ocean (La Réunion Island) since batch fecundity (based on the
424 weight of hydrated oocytes per ovary) and reproductive activity (based on period with a high
425 gonadal index) increased with female LJFL (Poisson and Fauvel, 2009). However, maternal
426 effects remain to be investigated for the Mediterranean swordfish, including from the point of
427 view of lipid and energy storage, as already observed for Pacific Bluefin tuna (Hiraoka et al.,
428 2019).

429 Energy acquisition from food, energy storage, and allocation for reproduction can be adaptive
430 responses to environmental conditions, which define the energy allocation strategy of a fish
431 species along the capital to income breeder continuum (McBride et al., 2015). For instance,
432 physiological needs during ovary maturation of yellowfin tuna *T. albacares* from the Western
433 Indian Ocean are thought to be insured by both prey selection of small fatty fish (Zudaire et al.,
434 2015) and previous energy storage in the liver (Zudaire et al., 2014), which classify this species

435 as an income-capital breeder (Zudaire et al., 2014). Such mixture of capital- and income-
436 breeding strategies is common in large pelagic fishes (Dhurmeea et al., 2018; Hiraoka et al.,
437 2019). To investigate the reproductive strategy of swordfish, lipid content of muscle and liver
438 should be analysed in association to gonads throughout the year, to detect whether or not lipids
439 are stored before reproduction.

440

441 **Swordfish gonad as a food source for human consumption**

442 The world health organization (WHO) recommends a daily intake of 250–500 mg of 22:6n-3 +
443 20:5n-3 for human health, especially to reduce oxidative damage of the brain and to improve
444 neurological outcomes (Butt and Salem, 2016; FAO/WHO, 2010). At the same time, 22:6n-3
445 is better assimilated when bounded to phospholipids rather than to TAG (Sugasini et al., 2019;
446 Tsushima et al., 2014). Swordfish gonads are rich in 22:6n-3-phospholipids, therefore, these
447 gonads are of a high nutritional interest for human health. Swordfish gonads are fatty tissues
448 that can however be contaminated by persistent and liposoluble organic pollutants. In particular,
449 polychlorobiphenyls (PCBs) and dichlorodiphenyl-trichloroethane (DDTs) are abundant
450 organic pollutants in tissues of the Mediterranean swordfish (Mehouel et al., 2021; Stefanelli et
451 al., 2004). Stefanelli et al. (2004) indicated that gonads are among the less contaminated tissues,
452 but ovaries are usually more contaminated than testes by both PCBs and DDTs (Stefanelli et
453 al., 2004). In the western Indian Ocean, perfluoroalkyl substances (PFASs) have been shown
454 to be particularly high in both swordfish ovaries and testes (Munsch et al., 2020). Regarding
455 trace metals, as most marine top predators, swordfish is mainly contaminated by mercury
456 (sometime over the safety limit set at 1 ppm ww by WHO), and in a lesser extent by arsenic,
457 while other heavy metals (cadmium, lead) are generally found within safety limits (Bodin et al.,
458 2017; Di Bella et al., 2020; Gobert et al., 2017; Sulimanec Grgec et al., 2020). Contamination
459 of swordfish gonads by trace metals remains to be assessed for the Mediterranean Sea
460 population. In the western Indian Ocean, it was shown that testes contain higher mercury levels
461 than ovaries, even if it was still within safety limits (Hollanda et al., 2017). Even if the gonads
462 of swordfish from Corsica Island have low concentrations of contaminants, which still needs to
463 be assessed, it should not justify an increase in the exploitation of this species, given the already
464 overfished stock. Few hundred of kg of swordfish gonads are 'wasted' yearly in Corsica Island
465 (i.e., wasted as human food source, but not for the ecosystem; Fuiman et al., 2015), and this
466 production of already fished fish should thus enter in short and local supply chains (e.g.,
467 restaurant, gourmet food).

468

469 Conclusion

470 As several large pelagic fish, swordfish gonads contain relatively low total lipids (1.7% ww).
471 However, while lipid class and FA concentrations remained unchanged during teste maturation,
472 concentrations of PC, TAG, 16:0, 18:1n-9, and 22:6n-3 followed “inverted U-shaped”
473 relationships with ovarian maturation. The energy allocation strategies and maternal effect
474 remain to be assessed for Mediterranean swordfish, requiring the analysis of other tissues, a
475 larger size range of females in spawning capable stages, and a year-round sampling. Swordfish
476 gonads from the Mediterranean Sea might also represent a good food source for human
477 consumption, but information on pollutant content is still missing and it should be assessed
478 before approval for human use.

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488

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Highlights

- Swordfish gonads contain <2% wet weight of total lipids
- Triacylglycerol and phosphatidyl choline are two main lipid classes of swordfish gonad
- Concentrations of the main lipid classes and FA increase in ovary until spawning
- 22:6n-3 and 18:1n-9 are selectively allocated to the ovarian maturation until spawning
- Swordfish gonad represents a valuable food source of omega-3 for humans

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Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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