

When size matters: The gonads of larger female yellowfin tuna (*Thunnus albacares*) have different fatty acid profiles compared to smaller individuals

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Abstract :

How the size of female yellowfin tuna (*Thunnus albacares*) affects their spawning capability and fecundity is still an open and unresolved question due to the difficulties in investigating these complex effects in highly migratory pelagic marine fish species. However, this information is key to understanding the reproductive potential and resilience of the stock. We investigate how energetic resources are allocated for reproduction by female yellowfin tuna according to their size in the Gulf of Guinea (central-eastern Atlantic Ocean). Our results reveal that larger females have not only larger ovaries by virtue of their greater abdominal cavity, but also different fatty acid profiles in the gonads compared to smaller females, with potential effects on their spawning and recruitment patterns. This study contributes to the knowledge of size-dependent variation in female yellowfin tuna and paves the way for future studies on size-dependent effects on reproductive parameters in this species.

Keywords : Tuna fishery, Maternal effect, Yellowfin tuna, Reproductive potential

42 Introduction

43 Understanding the productivity and resilience of fish stocks, which contribute to define the level of
44 fishing mortality they can sustain and their ability to recover from depletion, is crucial to provide sound
45 scientific advice for fishery management (Morgan *et al.*, 2009). However, the estimation of stock
46 productivity, which mainly relies on the stock–recruit relationship, is a difficult challenge in the study
47 of marine fish stocks’ dynamics and management (Myers *et al.*, 1998). The stock-recruitment
48 relationship is traditionally measured by estimating the spawning stock biomass and is used as a proxy
49 of stock reproductive potential (Trippel, 1999; Tomkiewicz *et al.*, 2003; Lowerre-Barbieri *et al.*, 2011).
50 Using spawning stock biomass for stock reproductive potential implies that the survival rates of
51 offspring are independent from parental age, body size and condition (Cardinale and Arrhenius, 2000),
52 and that the total egg production per unit weight is invariant over time (Morgan *et al.*, 2009). Thus,
53 spawning stock biomass does not take into account a variety of fundamental attributes, such as the
54 fecundity, atresia, duration of reproductive season, daily spawning behaviour and spawning fraction
55 (Murua *et al.*, 2003a).

56 Accurate knowledge of the reproductive characteristics that have a direct influence on the
57 productivity and resilience of commercial fish species is fundamental for developing effective and
58 realistic fishery management and conservation strategies (Trippel, 1999; Morgan *et al.*, 2009; Brown-
59 Peterson *et al.*, 2011). In this context, some concerns have been raised about the appropriateness of
60 spawning stock biomass (Marshall *et al.*, 1998; 1999) as a proxy of reproductive potential, which
61 assumes that fecundity is related to the weight-at-age of the sexually mature portion of the stock
62 irrespective of the demographic composition of adults (Murawski *et al.*, 2001; Kell *et al.*, 2015). This,
63 for instance, equates to claiming that many smaller mature individuals with the same weight of few
64 large mature individuals will produce the same amount of offspring. This means that first time
65 spawners will produce the same (and of the same quality) amount of eggs per weight than repeat
66 spawners. On the contrary, there is an increasing consensus in fishery science that spawning stocks
67 are composed by individuals with a range of sizes and ages that may contribute differently to spawning
68 and recruitment (Marshall *et al.*, 1998; Scott *et al.*, 1999, Kell *et al.*, 2015).

69 There is a general acknowledgment of the relevant impacts that “maternal effects” can have on
70 fecundity and viability of eggs and larvae (Kjesbu *et al.*, 1998; Scott *et al.*, 1999; Trippel, 1999; Berkeley

71 *et al.*, 2004). These effects include that larger females can allocate larger relative amounts of
72 reproductive resources for postnatal use. The positive relationship between the mothers' size/age and
73 both the potential productivity and the survival rates of the recruits has been demonstrated in other
74 fish species (Marshall *et al.*, 1998; 1999; Cardinale and Arrhenius, 2000; Shelton *et al.*, 2015; Berkeley
75 *et al.*, 2004; Bobko and Berkeley, 2004; Riveiro *et al.*, 2004). Moreover, older and larger females,
76 having a wider spatial and temporal window for spawning than smaller females, concomitant with
77 more spawning events in a season can enhance the perspectives for their larvae to encounter
78 advantageous conditions to survive (Birkeland and Dayton, 2005). In doing so, larger females should
79 invest a higher amount of energy for reproduction than smaller ones. This energy is mainly provided
80 by the metabolization of lipids and their constituent fatty acids, which represent the main energetic
81 resource in fish (Tocher, 2003).

82 Lipids can be divided into two main groups according to their chemical properties and functions: polar
83 lipids (PLs) and neutral lipids (NLs). PLs mainly correspond to the lipid class of phospholipids and to a
84 lesser extent to the ketones and wax-esters. PLs are important constituents of membranes and have
85 an important role as precursors in eicosanoid metabolism, i.e. structural fat. Contrary, NLs –
86 comprising triacylglycerols and sterols – serve primarily as depot fat, mainly used as an energy source.
87 Yet, the importance of fatty acids, specifically the omega-6 (ω 6) arachidonic acid (AA: 20:4n-6) and
88 those of the omega-3 (ω 3) type such as eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic
89 acid (DHA: 22:6n-3), in fish reproduction is well known (Watanabe, 1982). Particularly important are
90 also the polyunsaturated fatty acids (PUFAs), which are functionally essential for fish reproduction,
91 influencing egg quality, spawning, hatching and larval survival (Sargent *et al.*, 1989; 2002). PUFAs also
92 intervene in regulating the production of eicosanoids, steroid hormones and gonad development
93 (Izquierdo *et al.*, 2001). Hence, fatty acid composition in both NLs and PLs can provide major insight
94 for understanding fish energetic investment for reproduction.

95 The acquisition of lipids and their fatty acids relative to reproduction time follows two main strategies
96 in fish: 1) capital breeders, which store the required energy before the onset of reproductive period,
97 and 2) income breeders, which acquire it by feeding during the reproductive period (Murua *et al.*,
98 2003b; Alonso-Fernández and Sabórido-Rey, 2012; Aristizabal, 2007). In income breeders, the fatty
99 acid composition of the female gonad is greatly affected by dietary fatty acid content, which, in turn,
100 directly influences the egg quality in a short period of time (Izquierdo *et al.*, 2001). However, the
101 separation between these two strategies is not clear and there are gradual and mixed strategies
102 between them. A prime example of a species showing such a gradient in strategies is the yellowfin
103 tuna (*Thunnus albacares*; YFT), which is described as an income-capital breeder (Zudaire *et al.*, 2013b).

104 This species affords the cost of reproduction by both strategies, by feeding during the spawning period
105 and using energetic resources it has previously acquired. Despite the fundamental impact that an
106 understanding how size variation in YFT females affects their fecundity and reproductive potential
107 (including the quality of eggs) could have on stock resilience and, hence, management approaches,
108 very few studies to date have been conducted to understand how the size of YFT females affects their
109 reproductive potential (Zudaire *et al.*, 2014). This is mainly due to the operational difficulties (e.g.
110 sampling) and costs in investigating these complex effects in the wild.

111 Here, we aim to investigate whether the fatty acids profiles in the gonads of Atlantic YFT females are
112 correlated with their size, which in turn could contribute to variation in spawning and recruitment.
113 This study has also important implications in evaluating the potential age/size related females' larger
114 productivity in the Atlantic Ocean, where YFT is still considered for management purposes as a single
115 panmictic population (Pecoraro *et al.* 2017).

116

117 **Materials and Methods**

118 **Fish sampling**

119 Females of yellowfin tuna were caught by purse-seine vessels in the Gulf of Guinea (Eastern Atlantic
120 Ocean) from April 2013 to January 2014 (Fig.1). Morphometric measurements, reproductive stage
121 assessment and tissue sampling were carried out at the cannery "Pêche et Froid" of Abidjan, Ivory
122 Coast. For each fish, the fork length (F_L ; cm), the total fish weight (W ; kg) and gonad weight (W_G ; g)
123 were recorded. Each fish was assigned to a macroscopic maturity stage following the maturity
124 reference scale for this species (Diaha *et al.* 2015). For the purpose of the present study, we selected
125 50 spawning capable phase individuals (i.e., with late-maturing or ripe ovaries - for details see Zudaire
126 *et al.*, 2013a) in the F_L range of 125.8-154.5 cm (Fig. 2).

127 From each female, a cross section of the ovary of 4-5 cm was sampled between the middle and end
128 part of the right or left lobe and preserved in 4% buffered formaldehyde for further analyses. In
129 addition, a 2 g sample of gonads was collected and stored frozen in labelled microtubes for fatty acid
130 analysis.

131

132 **Reproductive analysis**

133 **Histological analysis**

Commentato [cp1]: The section on histological analysis is lacking details on the methods used (i.e. embedding material, section thickness, number of sections per individual sample, stain used, etc.).

134 Each ovary, being characterized by an asynchronous ovarian development, was classified according
135 to the most advanced oocyte stage present in the ovary (Murua and Motos, 2006), applying the
136 terminology proposed by Brown-Peterson *et al.*, (2011), and established for YFT in Zudaire *et al.*,
137 (2013a): (i) immature phase (primary growth stage [PG]); (ii) developing phase (cortical alveolar [CA],
138 primary vitellogenesis [Vtg1], and secondary vitellogenesis [Vtg2] stages); (iii) spawning-capable phase
139 (tertiary vitellogenesis [Vtg3], germinal vesicle migration [GVM], and hydration stages [HYD]), and (iv)
140 regenerating phase. For the purposes of this work, the 50 females were selected in spawning-capable
141 phase, containing oocytes in the stages Vtg3, GVM and HYD, i.e. the most advanced oocyte
142 development stages. Atresia was not assessed because the brine conservation process used in the
143 purse seines damages the follicle and chorion of the oocytes, making it difficult to accurately quantify
144 alpha-atresia (Zudaire *et al.*, 2013a).

145

146 Oocyte size-frequency **distribution**

147 A portion of the preserved ovary of 0.04 g (± 0.01 g) was collected and analysed for oocyte size-
148 frequency distribution. Tissue was placed into a filter with a mesh size of 125 μm and sprayed with
149 high pressure water to separate the oocytes from the connective tissue. The separated oocytes were
150 located on a gridded plate, photographed at the stereomicroscope with a digital camera and analysed
151 with the ImageJ free software (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda,
152 Maryland, USA, <http://rbs.info.nih.gov/ij/>, 1997-2012) to count and automatically measure the
153 diameter of all the oocytes. The number of developing oocytes (NDO) was calculated for the oocytes
154 in the developing phase described by a diameter size larger than the minimum threshold diameter of
155 CA oocytes estimated at 120 μm (Zudaire *et al.*, 2013b).

156

157 Batch fecundity **analysis**

158 The gravimetric method (Hunter *et al.*, 1989) was used to assess the batch fecundity (BF), which is the
159 number of oocytes spawned per batch during the actively spawning phase without presence of new
160 post-ovulatory follicles. This method consists of counting the total number of oocytes in the most
161 advanced maturation stage, i.e. GVM or hydrated oocytes. For this purpose, three tissue subsamples
162 of 0.1 g (± 0.01) were collected from each individual fixed ovary. Each subsample was placed on a slide
163 and covered with 3-4 drops of glycerin to make translucent the oocytes in the GVM and hydrated
164 oocytes (Schaefer, 1987). After oocyte counting, BF was estimated as the weighted mean density of
165 the three subsamples multiplied by the total weight of the ovary. In case that the coefficient of

Commentato [cp2]: The authors describe a method for conducting oocyte size-distribution that is not referenced and without apparent validation. For example, have the authors confirmed that spraying oocytes with high pressure water (without indication as whether it is freshwater or marine water) does not damage nor cause osmotic shock to the oocytes and, therefore, possibly affecting diameter measurements? Please provide a published reference and/or proper validation for this method

Commentato [cp3]: In the description of the gravimetric method used to estimate batch fecundity, please clarify whether (1) the ovarian subsamples were sectioned prior to the use of glycerin, (2) the glycerin-treated samples or sections were stained for contrast, and (3) only GVM and hydrated oocytes were counted.

166 variation among the three measurements was higher than 10%, more subsamples were taken until
167 reaching coefficient of variation threshold to decrease the uncertainty around the BF estimate To
168 estimate the relative batch fecundity (BF_{rel}), the value of BF was divided by the gonad-free weight of
169 the fish.

170

171 **Analysis of fatty acids**

172 Fatty acids analysis was performed following the same methodology as used in Bodin *et al.* (2014) and
173 Sardenne *et al.* (2016). First, ovarian samples were subjected to cryogenic grinding by using a mixer
174 mill MM400 Retsch® (Verder, France), obtaining a homogenized powder. From this, a subsample
175 (0.1±0.01g) was weighed under a nitrogen atmosphere and extracted following the method of Folch
176 *et al.*, (1957). An aliquot of the extracted sample was separated by adsorption chromatography on a
177 silica gel micro-column (Kieselgel 70 to 230 mesh, heated at 450°C and deactivated with 6% water).
178 Neutral and Polar lipids were eluted with 10 mL chloroform-methanol mixture (98:2 v/v) and 20 mL of
179 methanol, respectively. After adding a known amount of C23:0 fatty acid as internal standard, each
180 fraction was transmethylated at 100°C with 10 wt% boron trifluoride-methanol (Metcalf and Schmitz,
181 1961). The fatty acid methyl esters were analysed on a TRACE 1310 gas chromatograph equipped with
182 an on-column injector and a flame-ionization detector (GC-FID, Thermo Scientific). Compounds were
183 separated on a FAMEWAX™ column (30 m, 0.32 mm internal diameter, Restek) using helium as carrier
184 gas at a constant flow of 15 mL/min. The injector temperature was set at 225°C and the oven
185 temperature was raised from 130°C to 245°C at 2°C/min after a stationary phase at 130°C for 1 min.
186 Peaks were identified by comparing sample retention times to those of commercial standard mixtures
187 (Menhaden oil and Food Industry FAME Mix, Restek) with Xcalibur 2.2 software. Results were
188 expressed in % as the relative abundance of total identified compounds in each lipid fraction.
189 According to their degree of unsaturation (number of ethylenic or “double” bonds), fatty acids were
190 grouped and estimated in saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs) and
191 PUFAs.

192

193 **Multiple regression analysis**

194 A multivariate linear regression model was built for the 50 selected spawning capable YFT females in
195 order to understand out how their fatty acid profiles change according to their size.

Commentato [cp4]: Please provide the exact percentages of SFAs, MUFAs and PUFAs for NR and PL (shown in Fig. 3) in the text of the Results section as some of these values are missing.

196 A known problem with multiple regression model is the multi-collinearity effects that occur when the
197 predictor variables are too strongly correlated to each other, making the parameter estimates
198 unstable and difficult to interpret. The possible presence of multi-collinearity among explanatory
199 variables was investigated through variance inflation factors (VIF) calculations. All models were
200 implemented with the R statistical platform (R Core Team 2019) and VIF was calculated using the R
201 package car (Fox and Weisberg 2019). For this reason, we built different models for each group of
202 fatty acids (SFAs, MUFAs and PUFAs) and the polar and neutral fractions were tested separately.
203 Finally, the fatty acids from the two lipid fractions with a significant effect on the response variable
204 were aggregated together in the model using the Akaike Information Criterion (AIC) as an objective
205 statistical criterion in order to select the best model, i.e. the one that explains the most variability
206 (deviance) in the data. The stepwise function StepAIC in the R package MASS was used to show the
207 decrease in AIC when additional covariates were added. This allows for a trade-off between the
208 number of covariates and the deviance explained.

209

210 **Results**

211 **Reproductive analysis**

212 The mean and median of batch fecundity were estimated at 2.66 ± 0.9 and 2.66 ± 1.2 million oocytes
213 and of relative batch fecundity at 50.2 ± 17.6 and 48.5 ± 17.4 oocytes/g of gonad-free weight,
214 respectively (Supplementary Material1). None of these variables were significantly associated with
215 fish size. The mean gonad weight (W_G) was ± 1275.9 g, ranging from 655.5 g to 2305.3 g
216 (Supplementary Material1). The fish size had a significant effect ($P < 0.01$) on the total number of
217 developing oocytes and W_G , explaining 12% and 14.5 % of the variance, respectively. Both variables
218 showed an increase in relation with the fish size.

219

220 **Analysis of fatty acids**

221 For NL, SFAs were the most abundant group (44.5%), while in PL the most abundant groups were SFAs
222 and PUFAs (40.19% and 40.32%, respectively). Overall, NL contained a higher percentage of SFAs
223 (45.1%) and MUFAs (21.4 %) but a lower percentage of PUFAs (33.6%) than PL (respectively, 43%,
224 13.6% and 43.4%) (Fig.3). The fatty acid C16:0 (palmitic acid) was the most abundant SFA in both
225 fractions (28.8% in NL vs 29.59% in PL), followed by C18:0 (stearic acid; 9.54% NL vs 9.27% PL).
226 Regarding MUFAs, in each lipid fractions the most abundant fatty acid found was the C18:1n-9 with a

227 higher concentration in NL (ranged from 5.16 to 24.10%) than in PL (ranged from 7.72 to 17.58%). The
228 primary source of total PUFAs found in ovarian tissues were ω -3 fatty acids C20:5n-3
229 (eicosapentaenoic acid; EPA) and DHA. In NL, it was observed a much higher level of DHA (21.28%)
230 than eicosapentaenoic acid (EPA; 4.17%). The arachidonic acid (AA; C20:4n-6) was the most abundant
231 ω -6 PUFA in our samples.

232

233 **Multiple regression analysis**

234 The measures of all the reproductive outputs were related to the gonad weight. For this reason, the
235 gonad weight was chosen as the response variable for the multiple linear regression model. In this
236 model, the fork length (F_L) and the fatty acid profiles were used as explanatory variables:

$$237 \quad G \sim F_L + FA1 + FA2 + \varepsilon$$

238 where G is the gonad weight, F_L is the fish fork length, FA1 and FA2 are the fatty acids profiles, and
239 their ratios, from the neutral (NL) and polar (PL) fractions, respectively ε are the residuals.

240

241 **Polar lipids**

242 For polar lipids, no significant correlation was found among both SFAs and MUFAs groups, W_G and fish
243 size. Instead, significant correlations were found with some combinations of PUFAs as well as with
244 some ω 3: ω 6 ratios. For the PL fraction, a significant correlation ($P < 0.001$) was detected considering
245 as explanatory variables the sums of ω 3- and ω 6-PUFAs), with an adjusted coefficient of determination
246 r^2 of 0.415 (Supplementary Material2). The regression coefficient was positive (Estimate: 3.92) for ω 3-
247 PUFAs, while it was negative for ω 6-PUFAs (Estimate: -2.52; Supplementary Material2).

248 Exploring the effects of each PUFA of the polar fraction, we detected that among all the ω 3 and ω 6
249 fatty acids, only DHA (RC: 51.864), AA (RC: -389.214) and C18:2n-6 (RC: 798.591) had a significant
250 effect on the model, explaining together with the F_L around 50% of the variability in W_G (adjusted $r^2 =$
251 0.505, $P < 0.001$). The adjusted r^2 further increased to 0.581 ($P < 0.001$) by adding the specific interaction
252 of those fatty acids with the fish size to the model (Supplementary Material2).

253

254 **Neutral lipids**

255 In neutral lipids, our results showed that some specific SFAs, MUFAs and PUFAs significantly affected
256 the W_G variability.

257 Specifically, for SFAs, C17:0 and C16:0 explained together around 33% of the variation of the W_G in
258 relation to fish size (Supplementary Material3). Significant correlations ($P < 0.001$) were also found with
259 C18:1n-9 and C18:1n-7 (MUFAs), which explained 26% of the variance in the W_G (Table S3). The only
260 two PUFAs, in the neutral fraction, with a significant and positive effect on the model were C20:4n-3
261 and C18:3n-6 (adjusted $r^2 = 0.37$, $P < 0.001$; Supplementary Material3). For all the considered models,
262 VIF values were always lower than 10, thus suggesting the absence of severe multi-collinearity.

263

264 **Multiple regression model for the PUFAs of both fractions**

265 These two PUFAs C20:4n-3 and C18:3n-6 of the neutral fraction were added in the model, together
266 with the three significant PUFAs detected for the polar fraction (DHA, AA and C18:2n-3). However, the
267 effect of C18:2 n-6 was not significant ($P > 0.05$) and hence it was removed from the model. Running
268 the model with the other four fatty acids explained almost 57% (adjusted $r^2 = 0.569$) of the variability
269 of the gonad weight and all the explanatory variables considered had a significant effect on the model
270 ($P < 0.05$; Table 1). The addition of the interactions between PUFAs and fish size further increased the
271 percentage of variability explained (adjusted $r^2 = 0.668$; Fig. 4). Specifically, the interactions between
272 the fish size with DHA, C20:4n-3 and C18:3n-6 had a positive effect on the model. While AA was the
273 only PUFA with a negative effect on the model. **Overall, the interactions of the fish size with the PUFAs**
274 **have a significant effect on the model ($P < 0.05$)**

275 **Discussion and conclusions**

276

277 Using fish gonad weight, which is a good indicator of the individual reproductive effort, we show that
278 larger YFT females possess larger gonads, probably due to a greater abdominal cavity which enabling
279 the development of larger ovaries for holding eggs. Perhaps more importantly, we also demonstrated
280 for the first time that larger females have different fatty acid profiles in the gonads compared to
281 smaller individuals. Indeed, the dependence on body length of fatty acid profiles in the gonads
282 represents one of the most original results of our study. This is important as the variation in fatty acid
283 profiles across female size classes could result in variation in spawning quality, with larger females
284 potentially producing higher-quality offspring that can in turn improve chance of survival in larvae
285 through a decrease of development duration from embryogenesis to the first oral feeding (Fernández-
286 Palacios *et al.*, 2011). Previous studies have already highlighted that size and age can affect YFT

287 reproductive potential, with larger females exhibiting a higher spawning fraction in the Eastern Pacific
288 Ocean (Schaefer, 1998) and a longer spawning period in the western Indian Ocean (Zudaire *et al.*,
289 2013b). Interestingly, the relative batch fecundity of the YFT female in the Eastern Pacific Ocean
290 (Schaefer, 1998) was higher (67.3; range 4.9–174.0) than the one measured in this study in the Atlantic
291 Ocean (50.2; range 14.9–88.9).

292 The variation in the fatty acid profiles in the gonads of female YFT is particularly evident in certain
293 polyunsaturated fatty acids (PUFAs) especially in polar lipids (PLs). In these latter, the significant
294 correlation of dietary PUFAs with the gonad weight and the fish size emphasizes their functional
295 importance for YFT reproductive processes (Tocher, 2003). Our results also indicate that bigger
296 females with bigger gonads have a higher concentration of specific ω 3 and lower concentration of
297 specific ω 6 PUFAs in the polar fraction.

298 Tunas cannot synthesize *de novo* ω 3 and ω 6 PUFAs since they lack of the appropriate fatty acid
299 desaturase enzymes (Tocher, 2003). Thus, the proportion of the different PUFAs in the ovaries reflects
300 the amount consumed by feeding. Balance in the diet of both PUFAs ω 3 and ω 6 is an essential point
301 for optimizing fish reproductive success (Acharia *et al.*, 2000). PUFAs in general, and ω 3 in particular,
302 actively participate in gonad maturation, egg quality (Izquierdo *et al.*, 2001) and larval growth of fish
303 (Tulli and Tibaldi, 1997), regulating also the production of eicosanoids (prostaglandins), steroid
304 hormones and gonad development (ovulation; Izquierdo *et al.*, 2001). Our results indicate that larger
305 females have a higher concentration of docosahexaenoic acid (DHA, C22:6n-3) but a lower
306 concentration of arachidonic acid (AA, C20:4n-6), and linoleic acid (C18:2n-6). DHA is an essential fatty
307 acid that cannot be synthesized by fishes from the essential precursor alpha-linolenic acid (Riediger *et al.*
308 *et al.*, 2009). It has a specific structural role in nervous tissue (Sargent *et al.*, 1993) and high supply of
309 DHA available after the start of feeding supports the rapid development of membrane systems
310 (Tocher, 2003). In other fish species, it was observed a positive correlation between the
311 responsiveness to a visual stimulus and a higher concentration of DHA in the neural tissue in the head,
312 in relation with the larvae's size (Burns and Fuiman 2019). Our results confirm that usually tuna lipids,
313 including triacylglycerols and phospholipids, have higher levels of DHA than EPA in the neutral fraction
314 (Murase and Saito, 1996).

315 Overall, we detected a higher PUFAs ω 3/ ω 6 ratio in PLs than in NLs of larger YFT females. These results
316 show that an increase of gonad weight in larger females corresponds also to a decrease of arachidonic
317 acid (AA) and a consequent increase of EPA/AA ratio. Higher levels of EPA/AA ratio, which is crucial
318 for determining eicosanoid actions, have been associated with a superior resistance to infection in
319 several marine and freshwater species (Sargent *et al.*, 1995). Therefore, dietary intake of these fatty
320 acids can assume a relevant importance in YFT reproduction, even if there is no information about the

321 optimal intake of ω 3 to guarantee the highest spawning quality and reproductive success of this
322 species.

323 High levels of ω 3 in lipids and, in particular of DHA and EPA are a prerogative of tuna species (Murase
324 and Saito, 1996). For instance, the Pacific YFT showed a total amount of ω 3 PUFAs around 35% of total
325 fatty acids with DHA alone accounting for 25%-30% (Sunarya *et al.*, 1995). Although the relatively high
326 level of ω 3 for YFT females seems to be an intrinsic characteristic of tuna species, the higher amount
327 of ω 3 in the gonads of larger females measured in this study may indicate a quantitative change in the
328 energetic strategy of retaining/accumulating those fatty acids for reproduction (Tocher, 2003).

329 In the neutral fraction, we observed a higher level of palmitic acid (C16:0) for SFAs and oleic acid
330 (C18:1n-9) for MUFAs significantly correlated with the gonad weight and the size of the females. These
331 fatty acids have important quantitative and qualitative roles in structural phospholipids (Bell and Dick,
332 1991) and they can be biosynthesized *de novo* by fish as well as by all known organisms (Sargent *et*
333 *al.*, 1989).

334 YFT individuals rely on the schooling behaviour of their prey to facilitate feeding, forming groups of
335 individuals with different size and age. Most of the large volume tuna fisheries rely on their target
336 species' tendency to aggregate in schools. For this reason, when the females were caught, most likely
337 they were catching the same prey. Therefore, the differences in proportions of fatty acid profiles
338 between individuals highlighted in this study was not a result of the different maternally-derived
339 nutrients, as instead already indicated in other studies (Burns and Fuiman 2019). Clearly, the
340 ontogenetic shift in the relative proportion of different fatty acids identified in this study is distinct
341 from previous results focusing on overall investment of energy resources in this species. Indeed, a
342 previous study has shown that smaller YFT females invest more energetic resources for somatic
343 growth than larger ones (Zudaire *et al.* 2014). In this study, the authors showed a negative relationship
344 between the amount of total lipids in the muscle and the size of YFT females. Therefore, larger
345 mothers may switch the energy allocation from somatic to gonad growth for ensuring future
346 reproductive opportunities (Wiegand *et al.*, 2007). This size-related energy allocation strategy might
347 be linked to a much higher natural mortality rate in females with a $F_L > 130$ cm than in midsize
348 individuals (Hampton and Fournier, 2001).

349 Future effort is needed to determine the fatty acid composition in somatic tissues such as white
350 muscle and liver in order to understand how energy is transferred from those tissues to the gonads
351 during spawning seasons and events in relation to female size. This information is crucial to confirm
352 the size-related fatty acids composition pattern observed in the gonads of YFT females by the
353 dynamics of the somatic energy reserves during reproduction. According to the evidence of YFT

354 population structure detected among oceans (Pecoraro *et al.* 2016, 2017), further studies have to
355 investigate how the fatty acid profiles in the gonads of female YFT varies in relation to their size in
356 each ocean.

357 The use of the spawning stock biomass as a proxy of YFT stock reproductive potential by the tRFMOs
358 is still a subject of debate (Zudaire *et al.*, 2014). The reproductive contribution of large females should
359 be further investigated in order to assist the management framework of YFT in the Atlantic Ocean.
360 Additional demographic criteria accounting for the reproductive importance of larger and most
361 experienced spawners, will also contribute to a proper estimation of the reproductive potential of YFT
362 stocks.

363 In such a context, a shared effort among the different Regional fisheries management organisations
364 (RFMOs) would be key to optimize the sampling of large individuals, which is one of the most
365 challenging tasks for these pelagic fish species. Protecting those larger spawners may increase per
366 capita reproductive output (Kaiser *et al.*, 2007) and, hence, contribute to increase prospects of better
367 offspring survival. Conversely, increasing the mortality of larger and most experienced spawners might
368 relatively reduce in larger proportions the reproductive potential of the stock, which could also alter
369 the time and the location of spawning events decreasing the production and quality of eggs released.
370 As large YFT females could have a crucial relative reproductive value (Grey and Law, 1987), intensely
371 contributing to year class strength and surplus production under exploited conditions (Arlinghaus *et*
372 *al.*, 2010), their protection could be a potential management measure to ensure the sustainability of
373 YFT in the Atlantic Ocean.

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531 List of figures

532

533 Fig. 1_Geographic origin of the yellowfin females sampled for this study. Each dot indicates a purse
534 seine fishing set. Dots are plotted with some transparency to indicate an overlap of fishing seats
535 hidden due to overplotting issue.

536

537 Fig. 2_ Distribution by 10-cm length classes for the 50 female yellowfin tuna sampled.

538

539 Fig. 3_ Stacked bar chart with the different concentration (in %) of the three fatty acid groups (SFA,
540 MUFA and PUFA) in the neutral (NL) and polar (PL) lipid fractions.

541 Fig. 4_ Scatter plots for the results of the final multiple regression model. The gonad weight (W_G , g) is
542 the response variable and fork length (F_L , cm) and the interaction of the fatty acids (C20:4n-3, C18:3n-
543 6 for the NL in green and C22:6n-3, C20:4n-6 for PL in blue) with the FL are the explanatory variables.
544 All the variables were scaled to perform the multiple regression model. The solid three lines indicate
545 the mean regression line and they were chosen automatically by the R package ggeffects for each
546 facet individually. The shaded areas represent the 95% confidence level.

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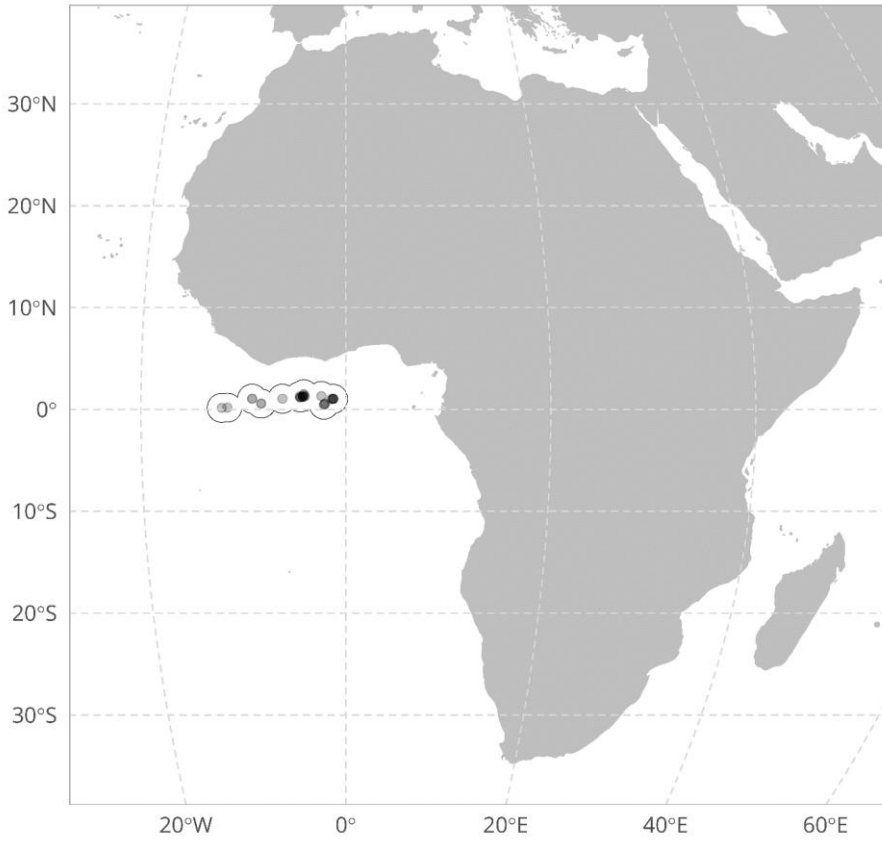
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549 List of Tables

550 Table 1_Summary of the regression coefficients of each variable included in the model. The total
551 adjusted r^2 and the p-value are also reported. PL: polar lipid fraction; NL: neutral lipid fraction. F_L : fork
552 length.

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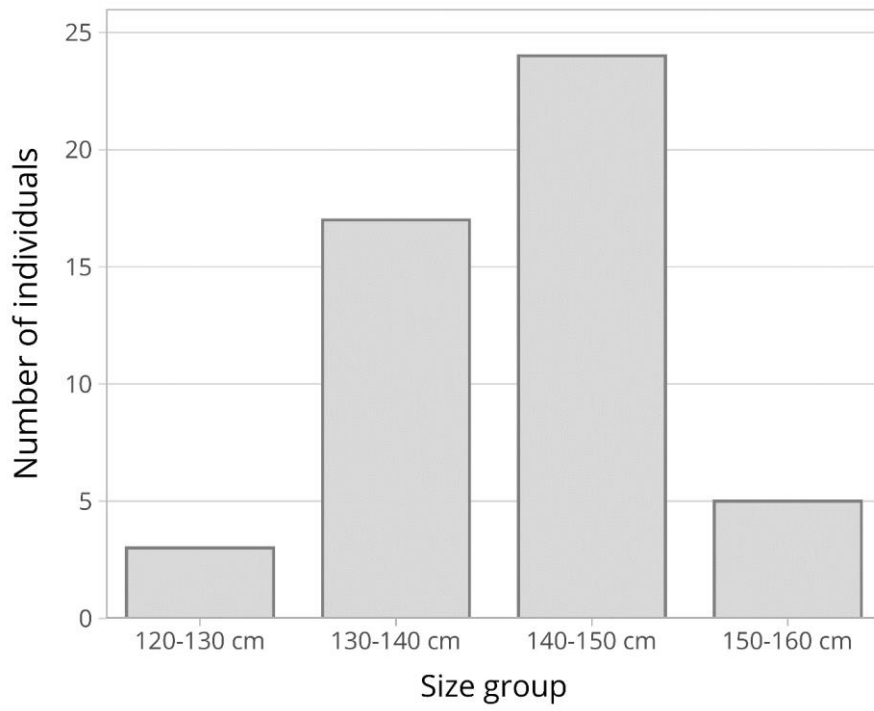


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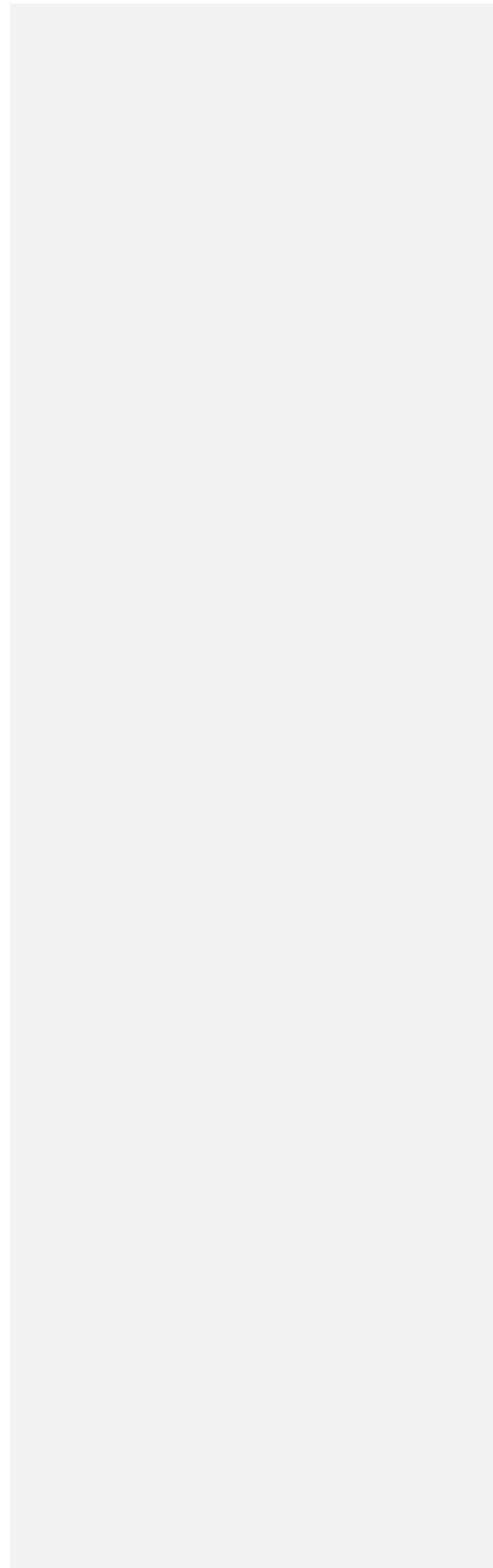
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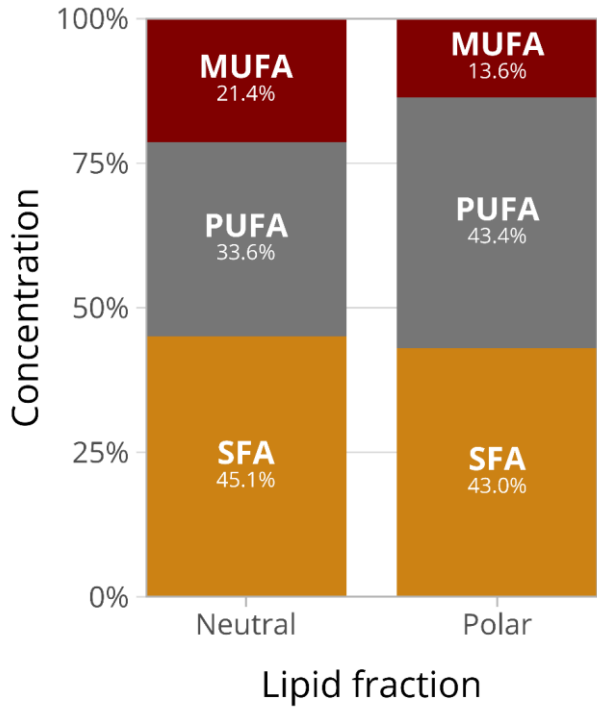
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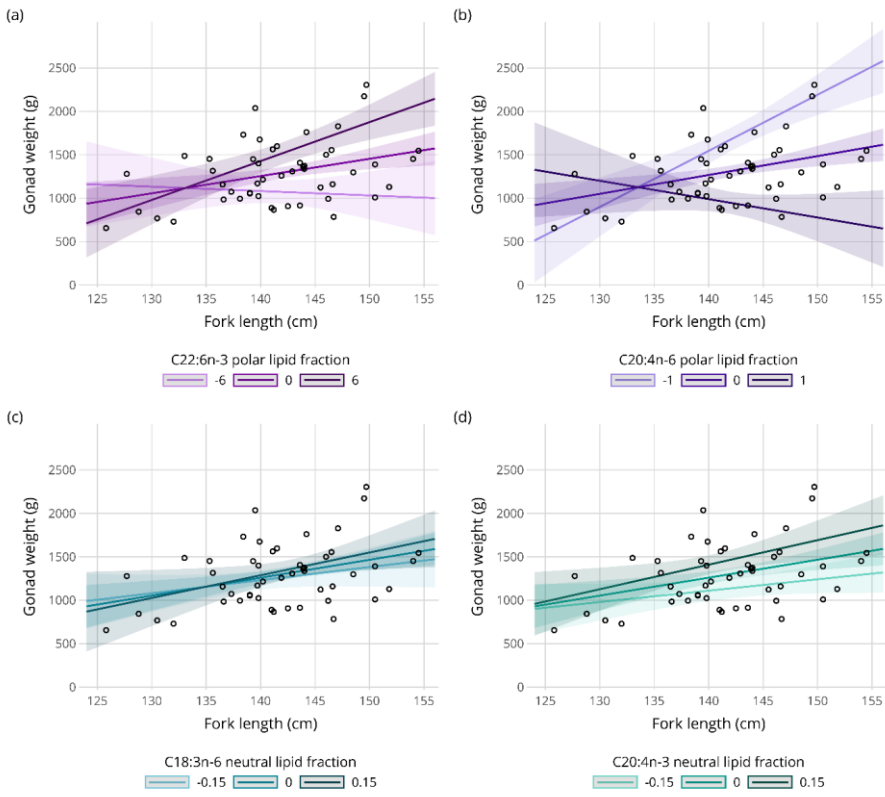
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| | Estimate | Std. Error | t value | p value | vif |
|-------------------------------|-----------------|-------------------|----------------|----------------|------------|
| (Intercept) | 5.539 | 33.971 | 0.163 | 0.871271 | |
| F_L | 20.779 | 5.618 | 3.699 | 0.000636 | 1.11 |
| C22:6n-3 PL | 32.088 | 10.971 | 2.925 | 0.005591 | 4.22 |
| C20:4n-6 PL | -321.181 | 65.992 | -4.867 | 1.72E-05 | 3.49 |
| C20:4n-3 NL | 1016.626 | 336.109 | 3.025 | 0.004283 | 2.15 |
| C18:3n-6 NL | 479.57 | 243.453 | 1.97 | 0.055634 | 1.24 |
| Adjusted r² | 0.5972 | | | | |
| p value | 5.21E-08 | | | | |

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