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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

T I

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).

There is a general acknowledgment of the relevant impacts that "maternal effects" can have on 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). This species affords the cost of reproduction by both strategies, by feeding during the spawning period and using energetic resources it has previously acquired. Despite the fundamental impact that an understanding how size variation in YFT females affects their fecundity and reproductive potential (including the quality of eggs) could have on stock resilience and, hence, management approaches, very few studies to date have been conducted to understand how the size of YFT females affects their reproductive potential (Zudaire *et al.*, 2014). This is mainly due to the operational difficulties (e.g. 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).

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

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

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 [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.

variation among the three measurements was higher than 10%, more subsamples were taken until reaching coefficient of variation threshold to decrease the uncertainty around the BF estimate To estimate the relative batch fecundity (BFrel), the value of BF was divided by the gonad-free weight of 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 (Metcalfe 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

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

219

220 Analysis of fatty acids

For NL, SFAs were the most abundant group (44.5%), while in PL the most abundant groups were SFAs and PUFAs (40.19% and 40.32%, respectively). Overall, NL contained a higher percentage of SFAs (45.1%) and MUFAs (21.4%) but a lower percentage of PUFAs (33.6%) than PL (respectively, 43%, 13.6% and43.4%) (Fig.3). The fatty acid C16:0 (palmitic acid) was the most abundant SFA in both fractions (28.8% in NL vs 29.59% in PL), followed by C18:0 (stearic acid; 9.54% NL vs 9.27% PL). Regarding MUFAs, in each lipid fractions the most abundant fatty acid found was the C18:1n-9 with a higher concentration in NL (ranged from 5.16 to 24.10%) than in PL (ranged from 7.72 to 17.58%). The primary source of total PUFAs found in ovarian tissues were ω -3 fatty acids C20:5n-3 (eicosapentaenoic acid; EPA) and DHA. In NL, it was observed a much higher level of DHA (21.28%) than eicosapentaenoic acid (EPA; 4.17%). The arachidonic acid (AA; C20:4n-6) was the most abundant ω -6 PUFA in our samples.

232

233 Multiple regression analysis

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

 $237 \qquad G \simeq F_L + FA1 + FA2 + \epsilon$

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

240

241 Polar lipids

For polar lipids, no significant correlation was found among both SFAs and MUFAs groups, W_G and fish size. Instead, significant correlations were found with some combinations of PUFAs as well as with some ω_3 : ω_6 ratios. For the PL fraction, a significant correlation (P<0.001) was detected considering as explanatory variables the sums of ω_3 - and ω_6 -PUFAs), with an adjusted coefficient of determination r² of 0.415 (Supplementary Material2). The regression coefficient was positive (Estimate: 3.92) for ω_3 -PUFAs, while it was negative for ω_6 -PUFAs (Estimate: -2.52; Supplementary Material2).

248Exploring the effects of each PUFA of the polar fraction, we detected that among all the ω3 and ω6249fatty acids, only DHA (RC: 51.864), AA (RC: -389.214) and C18:2n-6 (RC: 798.591) had a significant250effect on the model, explaining together with the F_L around 50% of the variability in W_G (adjusted $r^2 =$ 2510.505, P<0.001). The adjusted r^2 further increased to 0.581 (P<0.001) by adding the specific interaction</td>

252 of those fatty acids with the fish size to the model (Supplementary Material2).

253

254 Neutral lipids

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

Specifically, for SFAs, C17:0 and C16:0 explained together around 33% of the variation of the W_G in relation to fish size (Supplementary Material3). Significant correlations (P<0.001) were also found with C18:1n-9 and C18:1n-7 (MUFAs), which explained 26% of the variance in the W_G (Table S3). The only two PUFAs, in the neutral fraction, with a significant and positive effect on the model were C20:4n-3 and C18:3n-6 (adjusted r² = 0.37, P<0.001; Supplementary Material3). For all the considered models, 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 reproductive potential, with larger females exhibiting a higher spawning fraction in the Eastern Pacific Ocean (Schaefer, 1998) and a longer spawning period in the western Indian Ocean (Zudaire *et al.*, 2013b). Interestingly, the relative batch fecundity of the YFT female in the Eastern Pacific Ocean (Schaefer, 1998) was higher (67.3; range 4.9–174.0) than the one measured in this study in the Atlantic Ocean (50.2; range 14.9-88.9).

The variation in the fatty acid profiles in the gonads of female YFT is particularly evident in certain polyunsaturated fatty acids (PUFAs) especially in polar lipids (PLs). In these latter, the significant correlation of dietary PUFAs with the gonad weight and the fish size emphasizes their functional importance for YFT reproductive processes (Tocher, 2003). Our results also indicate that bigger females with bigger gonads have a higher concentration of specific w3 and lower concentration of specific w6 PUFAs in the polar fraction.

298 Tunas cannot synthesize de novo $\omega 3$ and $\omega 6$ 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 $\omega 3$ and $\omega 6$ is an essential point 301 for optimizing fish reproductive success (Acharia et al., 2000). PUFAs in general, and ω3 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 308 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 triacyglycerols 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 $\omega 3/\omega 6$ 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.

High levels of ω3 in lipids and, in particular of DHA and EPA are a prerogative of tuna species (Murase
and Saito, 1996). For instance, the Pacific YFT showed a total amount of ω3 PUFAs around 35% of total
fatty acids with DHA alone accounting for 25%-30% (Sunarya *et al.*, 1995). Although the relatively high
level of ω3 for YFT females seems to be an intrinsic characteristic of tuna species, the higher amount
of ω3 in the gonads of larger females measured in this study may indicate a quantitative change in the
energetic strategy of retaining/accumulating those fatty acids for reproduction (Tocher, 2003).

In the neutral fraction, we observed a higher level of palmitic acid (C16:0) for SFAs and oleic acid
(C18:1n-9) for MUFAs significantly correlated with the gonad weight and the size of the females. These
fatty acids have important quantitative and qualitative roles in structural phospholipids (Bell and Dick,
1991) and they can be biosynthesized *de novo* by fish as well as by all known organisms (Sargent *et 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 previous study has shown that smaller YFT females invest more energetic resources for somatic 342 343 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).

Future effort is needed to determine the fatty acid composition in somatic tissues such as white muscle and liver in order to understand how energy is transferred from those tissues to the gonads during spawning seasons and events in relation to female size. This information is crucial to confirm the size-related fatty acids composition pattern observed in the gonads of YFT females by the dynamics of the somatic energy reserves during reproduction. According to the evidence of YFT population structure detected among oceans (Pecoraro *et al.* 2016, 2017), further studies have to
investigate how the fatty acid profiles in the gonads of female YFT varies in relation to their size in
each ocean.

The use of the spawning stock biomass as a proxy of YFT stock reproductive potential by the tRFMOs is still a subject of debate (Zudaire *et al.*, 2014). The reproductive contribution of large females should be further investigated in order to assist the management framework of YFT in the Atlantic Ocean. Additional demographic criteria accounting for the reproductive importance of larger and most experienced spawners, will also contribute to a proper estimation of the reproductive potential of YFT 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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377 References

378	Acharia, K., Lal, B., Singh, T.P., and Pati, A.K. 2000. Circadian Phase Dependent Thermal Stimulation of
379	Ovarian Recrudescence in Indian Catfish, (<i>Clarias batrachus</i>), Biological Rhythm Research, 31:
380	125-135.
381	
382	Alonso-Fernández, A., and Sabórido-Rey, F. 2012. Relationship between energy allocation and

302 Alonso-remained, A., and Sabondo-Rey, F. 2012. Relationship between energy allocation and
 383 reproductive strategy in *Trisopterus luscus*. The Journal of Experimental Marine Biology and
 384 Ecology, 416: 8–16.

- Aristizabal, E.O. 2007. Energy investment in the annual reproduction cycle of female red porgy,
 (*Pagrus pagrus*)(L.). Marine Biology, 152: 713–724.
- Arlinghaus, R., Matsumura, S., and Dieckmann, U. 2010. The conservation and fishery benefits of
 protecting large pike (*Esox Lucius*)(L.) by harvest regulations in recreational fishing. Biological
 Conservation, 143: 1444–1459.
- Bell, M.V., and Dick, J.R. 1991. Molecular species composition of the major diacyl glycerophospholipids
 from muscle, liver, retina and brain of cod (*Gadus morhua*). Lipids, 26: 565–573.
- Berkeley, S.A., Chapman C., and Sogard, S.M. 2004. Maternal age as a determinant of larval growth
 and survival in a marine fish, *Sebastes melanops*. Ecology, 85: 1258–1264.
- Birkeland, C., and Dayton P.K. 2005. The importance in fishery management of leaving the big ones.
 Trends in Ecology & Evolution, 20: 356–358.
- Bobko, S.J., and Berkeley, S.A. 2004. Maturity, ovarian cycle, fecundity, and age-specific parturition of
 black rockfish (*Sebastes melanops*). Fishery Bulletin, 102: 418–429.
- Bodin, N., Lucas, V., Dewals, P., Adeline, M., Esparon, J., Chassot, E. 2014. Effect of brine immersion
 freezing on the determination of ecological tracers in fish. European Food Research and
 Technology, 238: 1057-1062.
- Brown-Peterson, N.J., Wyanski, D.M., Saborido-Rey, F., Macewicz, B.J., and Lowerre-Barbieri, S.K.
 2011. A standardized terminology for describing reproductive development in fishes. Marine
 and Coastal Fisheries, 3: 52–70.
- Burns, C.M., and Fuiman L. A. 2019. Maternally derived nutrients influence fatty acid composition and
 predator evasion behaviour of larval southern flounder, *Paralichthys lethostigma*. Journal of
 Experimental Marine Biology and Ecology, 514-515: 41-47.
- 407 Cardinale, M., and Arrhenius, F. 2000. The relationship between stock and recruitment: are the
 408 assumptions valid? The Marine Ecology Progress Series, 196: 305–309.
- Diaha, N.C., Zudaire, I., Chassot, E., Pecoraro, C., Bodin, N., Amandè, M.J., Dewals, P., Romeo
 M.U., Irié, Y.D., Barryga, B.D., Gbeazere, D.A., Kouadio, D. 2015. Present and future of
 reproductive biology studies of yellowfin tuna (*Thunnus albacares*) in the eastern Atlantic
 Ocean. Collective Volume of Scientific Papers ICCAT, 71 (1), 489-509. ISSN 1021-5212.
- Fernández-Palacios, H., Norberg, B., Izquierdo, M., and Hamre, K. 2011. Effects of Broodstock Diet on
 Eggs and Larvae. In Larval Fish Nutrition, G. J. Holt (Ed).
- Folch, J., Lees, M., and Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification
 of total lipids from animal tissues. The Journal of Biological Chemistry, 226: 497–509.
- Fox, J., and Weisberg, S. 2019. An R Companion to Applied Regression, Third edition. Sage, Thousand
 Oaks CA.
- 419 Grey, D.R., and Law, R. 1987. Reproductive values and maximum yields. Functional Ecology, Volume
 420 1, No. 4, pp. 327-330: 327–330.
- Hampton, J., and Fournier, D.A. 2001. A spatially disaggregated, length-based, age-structured
 population model of yellowfin tuna (*Thunnus albacares*) in the western and central Pacific
 Ocean. Marine Freshwater Research, 52: 937–963.

- Hunter, J.R., Macewicz, B.J., and Kimbrell, C.A. 1989. Fecundity and other aspects of the reproduction
 of sablefish, *Anoplopoma fimbria*, in central California waters. California cooperative oceanic
 fisheries investigations Reports, 30: 61–72.
- Izquierdo, M.S., Fernandez-Palacios, H., and Tacon A.G.J. 2001. Effect of broodstock nutrition on
 reproductive performance of fish. Aquaculture, 197: 25–42.
- Kaiser, M.J., Blyth-Skyrme, R.E., Hart, P.J., Edwards-Jones, G., and Palmer, D. 2007. Evidence for
 greater reproductive output per unit area in areas protected from fishing. Canadian Journal of
 Fisheries and Aquatic Sciences, 64: 1284–1289.
- Kell, L.T., Nash, R.D.M., Dickey-Collas, M., Mosqueira, I., and Szuwalski, C. 2015. Is spawning stock
 biomass a robust proxy for reproductive potential? Fish Fisheries, 17: 596-616.
- Kjesbu, O.S., Witthames, P.R., Solemdal, P., and Walker, M.G. 1998. Temporal variations in the
 fecundity of Arcto-Norwegian cod (*Gadus morhua*) in response to natural changes in food and
 temperature. Journal of Sea Research, 40: 303–321.
- Lowerre-Barbieri, S.K., Ganias, K., Saborido-Rey, F., Murua, H., and Hunter, J.R. 2011. Reproductive
 timing in marine fishes: variability, temporal scales, and methods. Marine and Coastal
 Fisheries, 3: 71–91.
- Marshall, C.T., Kjesbu, O.S., Yaragina, N.A., Solemdal, P., and Ulltang, Ø. 1998. Is spawner biomass a
 sensitive measure of the reproductive and recruitment potential of Northeast Arctic cod?
 Canadian Journal of Fisheries and Aquatic Sciences, 55: 1766–1783.
- Marshall, C.T., Yaragina, N.A., Lambert, Y., and Kjesbu, O.S. 1999. Total lipid energy as a proxy for total
 egg production by fish stocks. Nature, 402: 288–290.
- 445 Metcalfe, L.D., and Schmitz, A.A. 1961. The rapid preparation of fatty acid esters for gas 446 chromatographic analysis. Analytical Chemistry, 33: 363–364.
- 447 Myers, R.A., and Mertz, G. 1998. Reducing uncertainty in the biological basis of fisheries management
 448 by meta-analysis of data from many populations: a synthesis. Fisheries Research, 37: 51–60.
- Morgan, M.J., Murua, H., Kraus, G., Lambert, Y., Marteinsdottir, G., Marshall, C.T., O'Brien, L., and
 Tomkiewicz, J. 2009. The evaluation of reference points and stock productivity in the context
 of alternative indices of stock reproductive potential. Canadian Journal of Fisheries and
 Aquatic Sciences, 66: 404–414.
- 453 Murase, T., and Saito, H. 1996. The docosahexaenoic acid content in the lipid of albacore (*Thunnus* 454 *alalunga*) caught in two separate localities. Fisheries Science, 62: 634–638.
- 455 Murawski, S.A., Rago, P.J., Trippel, E.A. 2001. Impacts of demographic variation in spawning
 456 characteristics on reference points for fishery management. ICES Journal of Marine Science,
 457 58: 1002–1014.
- Murua, H., Kraus, G., Saborido-Rey, F., Witthames, P.R., Thorsen, A., Junquera, S. 2003a. Procedures
 to estimate fecundity of marine fish species in relation to their reproductive strategy. Journal
 of Northwest Atlantic Fishery Science, 33: 33–54.
- 461 Murua, H., Saborido-Rey, F. 2003b. Female reproductive strategies of commercially important fish
 462 species in the North Atlantic. Journal of Northwest Atlantic Fishery Science Vol. 33: 23-32.

- 463 Murua, H., and Motos, L. 2006. Reproductive strategy and spawning activity of the European hake
 464 (*Merluccius merluccius*) (L.) in the Bay of Biscay. Journal of Fish Biology, 69: 1288–1303.
- Pecoraro, C., Babbucci, M., Villamor, A., Franch, R., Papetti, C., Leroy, B., Ortega-Garcia, S., Muir, J.,
 Rooker, J., Arocha, F., Murua, H., Zudaire, I., Chassot, E., Bodin, N., Tinti, F., Bargelloni, L.,
 Cariani, A. 2016. Methodologic assessment of 2b-Rad genotyping technique for population
 structure inferences in yellowfin tuna (*Thunnus albacares*). Marine Genomics 25: 43–48.
- Pecoraro, C., Zudaire, I., Bodin, N., Murua, H., Taconet, P., Diaz-Jaimes, P., Cariani, A., Tinti, F., and
 Chassot, E. 2017. Putting all the pieces together: Integrating current knowledge of biology,
 ecology, fisheries status, stock structure and management of Yellowfin Tuna (*Thunnus albacares*). Reviews in Fish Biology and Fisheries, 27: 811-841.
- 473 Riediger, N.D., Othman, R.A., Suh, M., Moghadasian, M.H. 2009. A systemic review of the roles of n-3
 474 fatty acids in health and disease. Journal of the American Dietetic Association, 109: 668–679.
- 475 Riveiro, I., Guisande, C., Maneiro, I., and Vergara, A.R. 2004. Parental effects in the European sardine
 476 (*Sardina pilchardus*). Marine Ecology Progress Series, 274: 225–234.
- 477 Sardenne, F., Bodin, N., Chassot, E., Amiel, A., Fouché, E., Degroote, M., Hollanda, S.J., Pethybridge,
 478 H., Lebreton, B., Guillou, G., Ménard, 2016. Trophic niches of sympatric tropical tuna in the
 479 Western Indian Ocean inferred by stable isotopes and neutral fatty acids. Progress in
 480 Oceanography, 146: 75-88.
- 481 Sargent, J.R. 1989. Ether-linked glycerides in marine animals. Marine biogenic lipids, fats and oils, In
 482 CRC Press, pp. 176-193. Ed. by R., G. Ackman. Boca Raton, Florida.
- Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., and Tocher, D.R. 1993. The metabolism of
 phospholipids and polyunsaturated fatty acids in fish. Aquaculture: Fundamental and Applied
 Research, 43: 103–124.
- 486 Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., and Tocher, D.R. 1995. Requirement criteria for
 487 essential fatty acids. Journal of applied ichthyology, 11: 183–198.
- Sargent, J.R., Tocher, D.R., & Bell, J.G. 2002. The lipids. *In*: Fish Nutrition, 3rd edn, pp.181-257. Ed. By
 Halver, J.E., Hardy, R.W., Elsevier (Academic Press), San Diego, California.
- 490 Schaefer, K.M. 1987. Reproductive biology of black skipjack, (*Euthynnus lineatus*), an eastern Pacific
 491 tuna. Inter-American Tropical Tuna Commission Bulletin, 19: 166–260.
- 492 Schaefer, K.M. 1998. Reproductive biology of yellowfin tuna (*Thunnus albacares*) in the eastern Pacific
 493 Ocean". Inter-American Tropical Tuna Commission, 21: 201-272.
- 494 Scott, B., Marteinsdottir, G., and Wright, P. 1999. Potential effects of maternal factors on spawning
 495 stock-recruitment relationships under varying fishing pressure. Canadian Journal of Fisheries
 496 and Aquatic Sciences, 56: 1882–1890.
- 497 Shelton, A.O., Hutchings, J.A., Waples, R.S., Keith, D.M., Akçakaya, H.R., and Dulvy, N.K. 2015. Maternal
 498 age effects on Atlantic cod recruitment and implications for future population trajectories.
 499 ICES Journal of Marine Science, 72: 1769–1778.
- Sunarya, W., Fitriati M. and Mulyani H. 1995. The effect of season on fat content and fattyacid profile
 especially n-3 of yellowfin tuna. Res. Contrib. IX Session Indo-PacificFishery Commission
 Working Party on Fish Technology and Marketing. FAO Fish.Rep., 514 Suppl: 205–209.

- 503Tocher, D.R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in504Fisheries Science, 11: 107–184.
- Tomkiewicz, J., Tybjerg, L., and Jespersen, AA. 2003. Micro-and macroscopic characteristics to stage
 gonadal maturation of female Baltic cod. The Journal of Fish Biology, 62: 253–275.
- Trippel, E.A., 1999. Estimation of stock reproductive potential: history and challenges for Canadian
 Atlantic gadoid stock assessments. Journal of Northwest Atlantic Fishery Science, 25: 61–82.
- Tulli, F., and Tibaldi, E. 1997. Changes in amino acids and essential fatty acids during early larval rearing
 of dentex. Aquaculture International, 5: 229–236.
- 511 Watanabe, T. 1982. Lipids nutrition in fish. Comparative Biochemistry 73: 3-15.
- 512 Wiegand, M.D., Johnston, T.A., Leggett, W.C., Watchorn, K.E., Ballevona, A.J., Porteous, L.R.,
 513 Casselman, J.M. 2007. Contrasting strategies of ova lipid provisioning in relation to maternal
 514 characteristics in three walleyes (*Sander vitreus*) populations. Canadian Journal
 515 of Fisheries and Aquatic Sciences, 64: 700–712.
- 516

517 Zudaire, I., Murua, H., Grande, M., Korta, M., Arrizabalagaet, H., Areso, J.J., and Delgado-Molina, A.
518 2013a. Fecundity regulation strategy of the yellowfin tuna (*Thunnus albacares*) in the Western
519 Indian Ocean. Fisheries Research 138: 80–88.

- 520 Zudaire, I., Murua, H., Grande, M., and Bodin, N. 2013b. Reproductive potential of yellowfin tuna
 521 (*Thunnus albacares*) in the western Indian Ocean. Fisheries Bulletin, 111: 252–264.
- 522 Zudaire, I., Murua, H., Grande, M., Pernet, F., and Bodin, N. 2014. Accumulation and mobilization of
 523 lipids in relation to reproduction of yellowfin tuna (*Thunnus albacares*) in the Western Indian
 524 Ocean. Fisheries Research, 160: 50-59.
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531	List of figures
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533 534 535 536	Fig. 1_Geographic origin of the yellowfin females sampled for this study. Each dot indicates a purse seine fishing set. Dots are plotted with some transparency to indicate an overlap of fishing seats hidden due to overplotting issue.
537 538	Fig. 2_ Distribution by 10-cm length classes for the 50 female yellowfin tuna sampled.
539 540	Fig. 3_ Stacked bar chart with the different concentration (in %) of the three fatty acid groups (SFA, MUFA and PUFA) in the neutral (NL) and polar (PL) lipid fractions.
541 542 543 544 545 546 547	Fig. 4_ Scatter plots for the results of the final multiple regression model. The gonad weight (W_G , g) is the response variable and fork length (F_L , cm) and the interaction of the fatty acids (C20:4n-3, C18:3n-6 for the NL in green and C22:6n-3, C20:4n-6 for PL in blue) with the FL are the explanatory variables. All the variables were scaled to perform the multiple regression model. The solid three lines indicate the mean regression line and they were chosen automatically by the R package ggeffects for each facet individually. The shaded areas represent the 95% confidence level.
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549	List of Tables
550 551 552	Table 1_Summary of the regression coefficients of each variable included in the model. The total adjusted r^2 and the p-value are also reported. PL: polar lipid fraction; NL: neutral lipid fraction. F_L : fork length.











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	Estimate	Std. Error	t value	p value	vif
(Intercept)	5.539	33.971	0.163	0.871271	
FL	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 r ²	0.5972				
p value	5.21E-08				