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Accumulation and mobilization of lipids in relation to reproduction of yellowfin tuna (*Thunnus albacares*) in the Western Indian Ocean

Iker Zudaire[a,*], Hilario Murua[a], Maitane Grande[a], Fabrice Pernet[c], Nathalie Bodin[b]

[a] AZTI-Tecnalia, Marine Research Division, Herrera Kaia-Portu aldea z/g, Pasaia, Gipuzkoa 20110, Spain
[b] IRD – UMR 212 EME, Avenue Jean Monnet, BP 171, 34200 Sète, France
[c] Ifremer – Centre Bretagne, ZI de la Pointe du Diable, CS 10070, 29280 Brest, Plouzané, France

*: Corresponding author : Iker Zudaire, tel.: +34 660061499 ; fax: +34 946572555 ;
email address : iker.zuda@gmail.com

Abstract:

Total lipid content and lipid class composition were analyzed in gonads, liver and white muscle of yellowfin tuna (*Thunnus albacares*) throughout ovary development to understand its reproductive allocation strategy and to assess the relation between female condition and reproduction. A total of 112 females were collected onboard purse-seiner in the Western Indian Ocean from January to March 2009, from June to July 2009, and from April to May 2010. Gonads were characterized by highly variable total lipid contents ranging from 5 to 27 µg mg^{-1} of wet weight (ww) with a predominance of neutral lipids, mainly triacylglycerols (TAG) and sterol- and wax-esters. The different lipid classes in gonads described an accumulative pattern through the maturity process from immature to hydration phase. Total lipid content in liver varied from 10 to 21 µg mg^{-1} ww, and serves as fuel for yellowfin tuna reproduction. TAG and phospholipid deposits became depleted as the ovary developed, suggesting a transfer of lipids directly from liver to the oocytes during vitellogenesis. In contrast, muscle total lipid content was low and constant throughout ovarian development (2.5–6 µg mg^{-1} ww). Hence, yellowfin tuna can be defined as an income-capital breeder species for which the cost of reproduction depends mainly on concurrent energy income from feeding and only little on stored lipids. Besides, no significant relationship between gonad lipid composition and fecundity was found in females able to spawn. Finally, the influence of yellowfin tuna aggregation behaviour on reproductive female condition has been investigated: gonad total lipid contents were higher in females caught in free-swimming schools than in females caught under fish aggregating devices (FADs). However, these results did not clarify whether the influence of FADs on associated yellowfin tuna affects their reproductive capacity.

Keywords : Tropical tuna ; Lipid class composition ; Reproductive allocation strategy ; Income-capital breeder ; Fecundity ; FADs

1. Introduction

The reproductive process implies energetic and physiological costs that could endanger current reproduction as well as the survival of individuals and/or future reproduction (Stearns, 1992). A decrease in acquired energy prior to and/or during the spawning period could negatively impact on different reproductive traits such as maturation, fecundity, and egg quality and size (Henderson et al., 1996, Izquierdo et al., 2001, Kjesbu et al., 1998, Margulies et al., 2007, Sargent et al., 2002 and Wiegand et al., 2007). Lipids are the major metabolic energy resource involved in the reproductive process in fishes (Johnson, 2009), and can be (i) mobilized from storage tissues to the developing oocytes, (ii) acquired directly from food or (iii) de novo synthesized in the ovarian follicles (Wiegand, 1996). A major proportion of lipids are catabolized to provide metabolic energy required for reproductive processes, while some are transferred via serum as vitellogenin proteins (rich in polar lipids; referred to as Vtg) and very low-density lipoproteins (rich in neutral lipids; referred to as VLDL), and deposited as yolk reserves in the oocytes (Sargent, 1995).

Lipids dynamic through the reproductive cycle is related to the functions of each lipid class during reproduction (Norton and MacFarlane, 1999), and a decrease in their quantity could limit fish productivity (Henderson et al., 1996, Marshall et al., 1999 and Wiegand et al., 2007) and affect the viability of progeny (Rainuzzo et al., 1997). The different reproductive strategies adopted by the species also influence lipid acquisition in females (Alonso-Fernández and Saborido-Rey, 2012 and Aristizabal, 2007). Fishes with synchronous oocyte development are likely to acquire the energy they require prior to the reproductive period (i.e., capital breeders), whereas fishes with asynchronous oocyte development are more dependent on current energy income from feeding (i.e., income breeders) (McBride et al., 2013). The boundary between both strategies is not always so clear and a range of strategies could be developed by species (Houston et al., 2006). The

monitoring of different condition indices throughout the reproductive cycle has suggested that yellowfin tuna could act as an income-capital breeder (Zudaire et al., 2013b), which means that the cost of reproduction is mainly offset by current energy income due to feeding activity during the spawning period, although some of the invested resources could come from stored energy acquired before the reproductive period. Other factors like the environmental conditions, food availability and fish migration can also affect lipid content and, consequently, the reproductive potential of individual fish (Henderson et al., 1996, 1998; Izquierdo et al., 2001; Wiegand et al., 2007). The intensive use of fish aggregating devices (FADs) by tuna purse-seine fisheries has been hypothesized (Hallier and Gaertner, 2008; Marsac et al., 2000) that may have negative impacts on tuna movement and migration, leading them to low-quality habitats, with potential detrimental effects on individual and population productivity. Studies on tagged tunas (Dagorn et al., 2007; Schaefer and Fuller, 2010) have estimated a residence time of 3-8 days for tuna individuals associated to FADs. Yellowfin tuna has a high metabolic rate (Bertrand et al., 2002) and females are able to tune rapidly their reproductive effort to variation in the food availability (Margulies et al., 2007). Several studies have dealt with the assessment of these potential impacts that FADs could exert on different aspects of tuna biology (Dagorn et al., 2012; Jaquement et al., 2011), but none of them looked at the potential impact of FADs on lipid dynamics during reproduction.

Yellowfin tuna is a large epipelagic species widely distributed in the tropical and subtropical waters of the major oceans (Collette and Nauen, 1983). It is a highly migratory species, growing rapidly and with early maturation (Schaefer, 1998, 2001; Juan-Jordá et al., 2013). Yellowfin tuna exhibits indeterminate fecundity with asynchronous oocyte development (Zudaire et al., 2013a). Spawning occurs in multiple batches (Schaefer, 1996, 1998) all year round in tropical regions (Schaefer, 2001) and the spawning frequency for Pacific specimens was estimated around 1.5 days (McPherson, 1991; Schaefer, 1998). In the Western Indian Ocean, some studies identified two reproductive periods (Zudaire et al., 2013b; Stequert el al. 2001); the main one from November to March and a second peak in June, while others authors identified a single spawning peak during the main period (Stéquert and Marsac, 1989; Zhu et al., 2008). The mean batch fecundity in the Indian Ocean has been estimated to be 3.1 million oocytes (Zudaire et al., 2013b), and Schaefer (1996) revealed that a female yellowfin tuna in the Pacific Ocean would spawn the equivalent of about 3.5 times its body weight per year. The last assessment of the

Yellowfin carried out in the Indian Ocean estimated that the stock is not overfished and that overfishing is not occurring (IOTC, 2012).

Most of the previous studies on lipid dynamics during sexual maturation were conducted in temperate tunas (Mourente et al., 2002; Zaboukas et al., 2006), and only few studies were conducted on yellowfin tuna which mainly were focused on human nutritional requirements (Medina et al., 1995; Saito et al., 1996). To our knowledge, there is a lack of information regarding the lipid accumulation and mobilization process related with yellowfin tuna reproduction. Describing the role of each lipid class during oocyte development and analysing the relationships between lipids and fecundity could provide a better understanding of the reproductive potential of the yellowfin tuna stocks in the Western Indian Ocean (Marshall et al., 1999). Thus, the main purpose of this study was to describe the energy allocation strategy for reproduction by investigating total lipid content and lipid class composition and the process of their allocation during ovarian development in the muscle, liver and gonads of female yellowfin tuna from the Western Indian Ocean. The relationship between total lipid and lipid class concentrations in these tissues and fecundity was also investigated. Finally, the influence of school types (FADs vs. free-swimming schools) on yellowfin tuna condition was evaluated, and the possible consequences for reproductive potential were discussed.

2. Material and Methods

2.1. Field sampling

Sampling was carried out by scientific observers onboard commercial purse-seiner. Two surveys in 2009 (from January 21st to March 23rd and from June 5th to July 25th) and one survey in 2010 (From April 3rd to May 21st) were conducted in the Western Indian Ocean (Fig. 1). Each fish was measured (fork length, FL) to the nearest centimeter and weighed to the nearest tenth of a kilogram. Liver and gonad weights were recorded to the nearest gram. A 4–5-cm cross section of the gonads was cut in-between the middle/end part of the right or left lobe and preserved in a solution of 4% buffered formaldehyde for subsequent histological analysis to assess the maturation status and fecundity. For lipid analysis, 10-g samples of gonads, liver and white muscle from the dorsal part of the fish between the head and the first dorsal fin

were collected. Each sample was stored at -20°C onboard, and then transferred at -80°C to a laboratory until further lipid analysis.

Three condition indices, the gonadosomatic index (GSI), the hepatosomatic index (HSI) and Fulton's condition factor (K), were estimated as follows:

$GSI = (W_g / W_f) \times 10^2$

$HSI = (W_l / W) \times 10^2$

$K = (W / L^3) \times 10^2$

where W_g and W_l correspond to the gonad and liver weights (in grams) respectively, W_f is the gonad-free body weight and W is total weight of the fish (in grams). L is the fork length (in centimeters).

2.2. Histological analysis

The histological classification of yellowfin tuna ovaries followed the terminology established in Zudaire et al. (2013a). The ovaries were classified according to the most advanced oocyte stage present in the ovary: *immature phase* (IP), which includes oocytes in the primary growth stage (PG); *developing phase* (DP), which includes cortical alveoli (CA), primary vitellogenesis (Vtg1) and secondary vitellogenesis (Vtg2) oocyte stages; *spawning-capable phase* (SCP), which includes tertiary vitellogenesis (Vtg3), germinal vesicle migration (GVM) and hydration (HYD) stages; and *regenerating phase* (RP), which is characterised by the presence of late-stage atresia and a thicker ovarian wall than seen in immature fish.

2.3. Fecundity analysis

The batch fecundity (BF), i.e., the total number of oocytes released per batch, and the number of developing oocytes (NDO), i.e., the standing stock of yolked oocytes in the ovary (Murua and Motos, 2006), were estimated. The BF was determined using the gravimetric method (Hunter et al., 1989) by counting the oocytes at the most advanced stage of maturation (including GVM and HYD). For BF analyses, three subsamples of 0.1±0.01 g from each ovary were collected. BF was calculated as the weighted mean density of the subsamples multiplied by the total weight of the ovary. The NDO was estimated in ovaries at SCP, with Vtg3 as the most advanced oocyte stage. For NDO estimation, the minimum threshold of CA oocyte size was established at 120 μm by Zudaire et al. (2013a). The standing stock of yolked oocytes was counted using ImageJ free software (Rasband, W.S., ImageJ, U.S. National

Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2012) based on protocol described in Zudaire et al. (2013a).

2.4. Lipid class analysis

Each 10-g sample was first subjected to cryogenic grinding by using a mixer mill, MM400 Retsch® (Verder, France). This process had to be carried out as rapidly as possible to avoid lipid degradation. A subsample (0.1±0.001 g) of the homogenized powder was then weighed under a nitrogen atmosphere. Total lipids were extracted following a Folch method (Folch et al., 1957), spotted onto S-III Chromarods (Iatron Laboratories Inc., Tokyo, Japan), and finally separated into SE-WE, ketones (KET), TAG, free sterols (ST), acetone mobile polar lipids (AMPL) and PL (Parrish, 1999). Chromarods were scanned using an Iatroscan MK-VI (Iatron Laboratories) thin-layer chromatography-flame-ionization detector analyser (TLC–FID). Concentrations of lipid classes are expressed as μg mg^{-1} on a wet weight (ww) basis. Total lipid content (μg mg^{-1} ww) corresponds to the sum of lipid classes, and enables estimation of the relative contribution (%) of each lipid class to the total fat.

2.5. Statistical analysis

A two-way split plot ANCOVA was used to estimate differences in lipid class concentrations as a function of maturity stage and tissues (gonads, liver and muscle). Condition indices (GSI, HSI and K) and fork length were used as covariates. The unit of replication was the fish in which maturity stage was determined. The main plots were maturity stages and subplots were tissues. Here we used a mixed linear model, which models not only the means of our data but their variances and covariances. The need for covariance parameters arose because the experimental units (fish) on which the variables were measured were grouped into clusters and repeated measurements (tissue) were taken on the same experimental unit. The repeated option was applied to the term 'Tissue'. Where differences were detected, least-square means multiple comparison tests were used to determine which means were significantly different. A three-way split plot ANOVA was used to estimate differences in lipid class concentrations as a function of school types (FADs vs. free-swimming schools), maturity stage and tissues. The main plots were school types and maturity stages and subplots were tissues. The repeated option was applied to the term 'Tissue'. Only females with ovaries at DP and SCP were included in this model to focus on mature females involved in the current reproductive process. Due to the absence of females with hydrated ovaries

178 (HYD) collected under FADs, this stage was excluded from the model. Finally, the relationships between
179 fecundity and condition indices and lipid classes were investigated using stepwise regression models. For
180 all statistical analyses, residuals were screened for normality using the expected normal probability plot.
181 When necessary, data were log+1 transformed to achieve normality of residuals and homogeneity of
182 variances. Homogeneity of variance–covariance matrices was graphically assessed. Analyses were carried
183 out using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA).
184
185 **3. Results**
186
187 A total of 112 yellowfin tuna were sampled: 67 females were caught under FADs and 45 females in free-
188 swimming schools. The fishes caught at FADs ranged from 50 to 120 cm FL and a total weight from 2.4
189 to 33.0 kg and fishes caught in free-swimming schools ranged from 56 to 153 cm FL and a total weight
190 from 3.7 to 63.9 kg (Table 1). Table 2 describes the numbers of sampled females, the fish size range (cm),
191 as well as the mean values (± standard error, SE) of the condition indices (GSI, HSI and K) and the
192 concentrations of total lipids in the three tissues (gonads, liver and muscle) by ovarian development
193 phases. Overall, the highest contents of total lipid in gonads were observed in females at the spawning-
194 capable phase (26.7±0.2 µg mg^{-1}), while the lowest values were found in ovaries of developing-phase
195 females (4.8±0.1 µg mg^{-1}). Liver exhibited the opposite pattern, with the highest total lipid contents in
196 immature phase females (20.8±0.1 µg mg^{-1}) and the lowest in females at the spawning-capable phase
197 (10.3±0.4 µg mg^{-1}). Gonads and liver exhibited around threefold and fourfold higher total lipid contents
198 than muscle, in which the highest and lowest total lipids were found in immature phase (5.9±0.1 µg mg^{-1})
199 and spawning-capable phase females (2.9±0.5 µg mg^{-1}), respectively.
200
201 *3.1. Lipid class composition along the reproductive cycle*
202 All females presented in the Table 2 were selected for the analysis of the lipid class composition along the
203 reproductive cycle. The outputs of mixed ANCOVA model described weak but significant positive
204 relationships between FL and TAG concentrations ($r^2 = 0.121$; $P<0.05$), as well as between FL and
205 TAG/ST ratio ($r^2 = 0.225$; $P<0.05$) in yellowfin gonads (Fig. 2A and 2B, respectively). Similarly, a
206 positive relationship between HSI and TAG/ST ratio ($r^2 = 0.109$; $P<0.05$) was observed in liver (Fig. 2D).
207 In contrast, a negative relationship was observed in the muscle between total lipids and FL ($r^2 = 0.196$;

208 $P<0.05$) (Fig. 2C). The interaction of Maturity × Tissue was significant ($P< 0.05$) for all the different
209 lipid classes, except ketones (Table 3); which indicate that different lipid classes in liver, muscle and
210 gonad varies with maturity stage (further described below).
211
212 In gonads, TAG concentrations showed a clear accumulative pattern through maturation (Fig. 3A). While
213 PG and CA stages exhibited low values (1.3±0.2 µg mg^{-1} and 0.6±0.1 µg mg^{-1}, respectively), TAG
214 increased significantly during vitellogenesis to reach the maximum levels in Vtg3 (7.3±0.2 µg mg^{-1}) and
215 GVM (7.5±0.5 µg mg^{-1}). Then, TAG decreased drastically, leading to concentrations in the regenerating
216 phase (1.1±0.2 µg mg^{-1}) as low as in the immature phase. Unlike in ovaries, TAG showed a pattern of
217 depletion in the liver through maturation (Fig. 3A). TAG levels decreased from PG (7.8±0.2 µg mg^{-1}) to
218 GVM (1.7±0.5 µg mg^{-1}), and then increased significantly in the hydration stage (7.0±0.6 µg mg^{-1}) to
219 reach maximum values in the regenerating phase (7.9±0.2 µg mg^{-1}). As regards yellowfin tuna muscle,
220 ovarian maturation had no effect on TAG (Fig. 3A).
221
222 SE-WE followed the same patterns as TAG in yellowfin tuna gonads and muscle throughout maturation
223 (Fig. 3B). Muscle SE-WE concentrations were not significantly affected by ovarian maturation, while
224 gonad SE-WE values showed an accumulative pattern, that is, low values in PG and CA stages (0.2±0.1
225 µg mg^{-1} and 0.3±0.1 µg mg^{-1}, respectively), followed by a significant increase over vitellogenesis to reach
226 the maximum values in Vtg3 (5.1±0.2 µg mg^{-1}) and GVM (5.2±0.4 µg mg^{-1}), and finally a drastic
227 decrease during hydration (0.4±0.5 µg mg^{-1}) and regenerating phases (0.2±0.2 µg mg^{-1}). SE-WE showed
228 the opposite trend to TAG in the liver (Fig. 3B): concentrations were low and relatively constant from PG
229 (1.6±0.1 µg mg^{-1}) to Vtg3 (1.5±0.2 µg mg^{-1}), then increased significantly in GVM (4.9±0.4 µg mg^{-1}) to
230 decrease again in hydration (2.9±0.5 µg mg^{-1}) and further in the regenerating phase (0.7±0.2 µg mg^{-1}).
231
232 PL also showed an accumulative pattern in the gonads (Fig. 3C) with a significant increase over the
233 vitellogenic process from Vtg1 (1.9±0.1 µg mg^{-1}) to Vtg3 (7.6±0.1 µg mg^{-1}), and a decrease from GVM
234 (6.6±0.3 µg mg^{-1}) to the regenerating phase (2.2±0.1 µg mg^{-1}). In the liver, PL decreased significantly
235 from CA (2.8±0.1 µg mg^{-1}) to GVM (0.9±0.3 µg mg^{-1}), while concentrations increased during hydration
236 (3.6±0.3 µg mg^{-1}) to finally decrease again in the regenerating phase (2.6±0.1 µg mg^{-1}). In the muscle, PL
237 concentrations decreased significantly from Vtg1 (3.3±0.1 µg mg^{-1}) to hydration (1.0±0.3 µg mg^{-1}), then

238 increased in the regenerating phase (2.5±0.1 µg mg^{-1}) to reach levels similar to those in the immature
239 phase (2.8±0.1 µg mg^{-1}) (Fig. 3C).
240
241 Despite very low levels, ST and AMPL in yellowfin tuna gonads followed the same pattern as the other
242 lipid classes (Fig. 3D and 3E). No significant effect of ovarian maturation was noted for ST and AMPL
243 concentrations in both yellowfin liver and muscle.
244
245 *3.2. Lipid class composition regarding fecundity estimates*
246 For BF analysis 9 females, ranging in size between 123 and 146 cm FL, were selected from the pool of
247 total samples. All of them were caught in free-swimming schools during the spawning period in 2009.
248 The estimated BF for these females ranged between 0.5 and 4.8 million eggs and the GSI was estimated
249 in 1.6±0.4, HSI in 0.9±0.2 and K in 1.7±0.1. For NDO analysis 18 females were selected, ranging in size
250 from 88 to 145 cm FL; 13 were caught in free-swimming schools and 5 under FADs, all of them during
251 the surveys performed in 2009. The estimated NDO ranged between 0.6 and 25.7 million oocytes. The
252 GSI for these females was estimated in 1.1±0.4, HSI in 0.8±0.2 and K in 1.9±0.2.
253
254 According to the stepwise regression analysis between BF and the condition indices (Table 4), only GSI
255 was significantly correlated with BF ($r^2 = 0.75$, $P< 0.05$); regarding NDO, none of the condition indices
256 met the required 0.05 level of significance to get into the model. Investigation of the influence of the
257 tissue lipid composition on yellowfin tuna fecundity revealed that only TAG and SE-WE concentrations
258 in the muscle were related to BF, explaining 86% of the variation (Table 4), while in gonad and liver none
259 of lipid classes met the required 0.05 level of significance to get into the model. In contrast, the stepwise
260 regression analysis between lipid composition and NDO for the three tissues showed that only ST was
261 significantly correlated to NDO in gonads ($r^2 = 0.40$, $P< 0.05$; Table 4).
262
263 *3.3. Lipid class composition regarding school types (FADs vs. free-swimming schools)*
264 From the total pool of samples, 39 females were selected to assess lipid class composition regarding the
265 school types (Table 5): 25 females, ranging in size between 122 to 148 cm FL and in weight between 31
266 and 63 kg, were caught in free-swimming schools and 14 females, ranging in size between 69 and 117 cm
267 FL and in weight between 8.4 and 29 kg, were caught under FADs. As shown in Table 6 and Fig. 4, the

mixed ANCOVA model revealed a significant interaction between tissue and school type only for total lipids, PL and AMPL. In particular, higher total lipid and PL concentrations were measured in the gonads of female yellowfin tuna caught in free-swimming schools (total lipids = 18.9±0.1 µg mg^{-1}; PL = 6.1±0.1 µg.mg^{-1}) than in females caught under FADs (total lipids = 12.6±0.2 µg mg^{-1}; PL = 3.3±0.1 µg mg^{-1}) (P< 0.05) (Fig. 4). In contrast, the school type did not seem to affect the liver and muscle lipid compositions. As regards AMPL, a significant difference of liver concentrations was noted between females caught in free-swimming schools (1.7±0.1 µg mg^{-1}) and those caught under FADs (1.0±0.1 µg mg^{-1}), while no effect was observed for gonads and muscle.

4. Discussion

The life history strategy of yellowfin tuna has been described as "opportunistic" (Essington, 2003; Fromentin and Fonteneau, 2001; Juan-Jorda et al., 2013) due to its early maturation, estimated around 75 cm FL in the Western Indian Ocean (L_{50}, Zudaire et al., 2013b), rapid growth and high reproductive output (Schaefer et al., 2001). Accomplishing such a reproductive effort requires a high energetic investment, and thus lipids and their constituent fatty acids are an essential source of nutrition providing a significant amount of metabolic energy and structural components for developing oocytes, similar to other tuna species (Mourente et al., 2002; Zaboukas et al., 2006). Saito et al. (1996) described that lipid composition of yellowfin tuna is mainly affected by the lipids of its prey and the environmental temperature (Saito et al., 1996). Despite the sampling strategy was fishery-dependent, female yellowfin tunas were collected covering the main two reproductive periods (Zudaire et al., 2013b). However, due to actual piracy problems along the study area, the sampling was conducted in two consecutive years, which could add inter-annual variability in terms of changes in environmental and tropich conditions affecting in some extend the results of the study.

4.1. Lipid class composition along the reproductive cycle

During fish ovarian development, the oocytes incorporate compounds essential for egg viability and larval survival (Rainuzzo et al., 1997). In yellowfin tuna ovaries, all lipid classes (TAG, SE-WE, PL, ST and AMPL) showed the same accumulative pattern through vitellogenesis; however, the observed variability in the deposition of each lipid class may reflect their function during ovarian development (Johnson, 2009). Similar to other fish species with oocytes containing oil globules (Kaitaranta and

298 Ackman, 1981; Mourente et al., 2002; Ortega and Mourente, 2010), yellowfin tuna ovaries described a
299 predominance of neutral lipids (mainly TAG and SE-WE) over the polar lipids (PL and AMPL),
300 comprising 62% and 65% of the total lipids in the ovaries in Vtg3 and GVM stages, respectively. This
301 pattern is related to the role of neutral lipids as metabolic energy resources for oocyte formation.
302 Phospholipids also contributed significantly to total lipids in yellowfin tuna gonads (around 33%) since it
303 plays a major role in cellular membranes and tissue formation (Tocher, 2003). Indeed, sufficient
304 quantities of both polar and neutral lipids are essential endogenous energetic resources for embryogenesis
305 and larvae development (Ortega and Mourente, 2010). However, in the present study, the gonads of
306 female yellowfin showed low total lipid contents (maximum mean value of 2.5% of gonad wet weight) in
307 comparison to other scombrids, being at least twofold lower than values observed in *Thunnus tonggol*
308 (Intarasirisawat et al., 2011), *Sarda orientalis* (Osako et al., 2009) and *Katsuwonus pelamis* (Hiratsuka et
309 al., 2004), and nearly fourfold lower than in *Sarda sarda* (Zaboukas et al., 2006). Species with
310 asynchronous oocyte development exhibit relatively low body energy investment in gamete formation
311 (Aristizabal, 2007), which explain the low observed gonad lipid contents regarding the high reproductive
312 output during the spawning season of yellowfin tuna (Zudaire et al., 2013b).
313
314 The decline of concentrations of the different lipid classes observed in the gonads at the hydration stage
315 probably results from a combination of two factors. First, the size of the oocytes increases significantly,
316 partly due to oocyte hydration (Wallace and Selman, 1981), a process that might contribute to the dilution
317 of the lipids in the ovaries. Indeed, a significant increase of the water content in the yellowfin tuna gonads
318 was observed between GVM (71.6±2.3%) and hydration stages (78.2±1.2%). Moreover, the process of
319 protein uptake into oocytes (i.e., Vtg and VLDL), which is also responsible for the lipid accumulation
320 stops abruptly at the time of germinal vesicle breakdown (Wallace and Selman, 1985).
321
322 The liver plays an important role in fish gonad development, being responsible for large lipid and fatty
323 acid deposits in the ovary, which are derived from prey and mobilized from the muscle during the
324 vitellogenesis process (Rinchard and Kestemont, 2003; Wiegand, 1996). When the liver is stimulated by
325 the ovary via estrogens, it starts to synthesise and secrete the two major sources of oocyte lipids: Vtg and
326 VLDL (Wiegand, 1996). In female yellowfin tuna, the liver showed two different patterns of lipid
327 acquisition and mobilization: TAG and PL showed depletion throughout ovarian development until the

GVM stage, while SE-WE seemed to accumulate between the Vtg3 and GVM stages. Before vitellogenesis occurred, the liver acted as a storage tissue for TAG and PL, with those lipid classes representing 40% and 19% of total lipids in the liver, respectively. Hence, a large proportion of liver TAG that was depleted through ovary development might have been transferred to the oocytes during vitellogenesis and the GVM stage, which would explain the simultaneous increment of TAG found in gonads during the maturation process. A similar process probably occurred with PL, although the largest reduction was observed during the first stages of ovary maturation. The mobilization of PL from the liver before vitellogenesis (between PG and Vtg1 stages) could correspond to a requirement of structural compounds during early oocyte formation and/or energy substrate utilization for specific lipoprotein synthesis, as previously noted in *Sparus aurata* (Almansa et al., 2001). On the other hand, the SE-WE accumulative pattern could be related to an intensive lipid requirement of gonads due to the continuous oocyte recruitment during the reproductive season; such an observation has already been made for the multiple-batch spawner cyprinidae fishes (Rinchard and Kestemont, 2003). However, SE-WE increase coincides with a major depletion of TAG at the GVM stage, and it could be hypothesized that the variations of these two lipid classes are interrelated. A similar result was described by Norton and MacFarlane (1999) for yellowtail rock (*Sebastes flavidus*) fish, in which the increase of PL was due to a depletion of TAG in the liver during the vitellogenesis process.

The white muscle of female yellowfin tuna was characterized by very low total lipid contents (maximum mean value of 0.6% wet weight in PG stage females), which is in accordance with previous estimations for this species (Medina et al., 1995; Saito et al., 1996) as well as other types of tuna such as *Thunnus obesus*, *Auxis thazard* and *Katsuwonus pelamis* (muscle total lipids: 0.8%, 0.6% and 0.8%, respectively; Medina et al., 1995), as well as *Sarda orientalis* (muscle total lipids: 0.8%–0.9%; Osako et al., 2009). No significant variations of lipid class concentrations were noted in the muscle through the reproductive cycle, with the exception of PL, which decreased during vitellogenesis. In contrast, other types of tuna like bluefin tuna (*Thunnus thynnus*) (Mourente et al., 2002) and bonito (*Sarda sarda*) (Zaboukas et al., 2006) have been described to accumulate larger lipid deposits in somatic tissues (muscle, mesenteric perigonadal tissue), with a predominance of neutral lipids. In these temperate species, the stored lipid reserves are devoted either to cover the requirements of high metabolic activity or for reproduction, corresponding to the particular energy allocation strategy used.

Yellowfin tuna exhibited a low capacity for energy storage through the reproductive cycle and showed very low lipid contents in gonads compared to other tunas, which can be a sign of a strategy of low energy allocation of this tuna species for the reproduction. . Yellowfin tuna exhibits indeterminate fecundity and asynchronous oocyte development (Zudaire et al., 2013a), and females feed intensively throughout the reproductive cycle (Itano, 2000; Zudaire in prep). All these lines of evidence together with the absence of a substantial endogenous source of energy for ovarian development make this species a suitable candidate to be considered as a pure income breeder (McBride et al., 2013; Alonso-Fernández et al., 2012). However, the lipid allocation pattern shown by the liver, accumulating certain lipids (TAG and PL) prior to vitellogenesis, could suggest that yellowfin tuna exhibits an intermediate strategy (income-capital breeder) in which, despite the priority of energy allocation from feeding, the energy stored before reproduction is also required for successful reproduction (Henderson and Morgan, 2002). Species that feed intensively through ovarian maturation incorporate more dietary lipids for oocyte development (Johnson, 2009), and thus the availability of food during the spawning season could greatly modulate the reproductive investment and condition the manner in which stored energy is used to offset the cost of reproduction (Aristizabal, 2007).

The length of yellowfin tuna exhibited positive relationships with TAG and TAG/ST ratio in the ovaries, although the coefficients of determination for both relationships were low. The increase of lipid concentration with female size was previously described for other fish species in which the size of the female could boost the lipid deposition to oocytes (Wiegand et al., 2007). Moreover, a significant negative relationship was observed between total lipid and FL in muscle. These patterns observed in gonads and muscle could explain the physiological changes and associated energy requirements and partitioning during the ontogeny of yellowfin tuna reflected in different reproductive allocation patterns, and they might be related to the energy balance between somatic growth and gonadic growth, which is size-dependent (Claramunt et al., 2007). Thus, a larger amount of lipid in the muscle of smaller (younger) individuals could reflect an increase in energy investment for somatic growth, while older and larger females could invest higher levels of energy through lipid reserves for gonadic growth as future reproductive opportunities decline (Wiegand et al., 2007).

388 *4.2. Lipid class composition regarding fecundity estimates*

389 Maternal condition and quantity of reserves in particular, are believed to limit reproductive potential
390 through fecundity in some species (Johnson, 2009; Marshall et al., 1999; Wiegand et al., 2007). Studies
391 with cultured (Margulies et al., 2007) and wild (Itano, 2000) female yellowfin tuna provided evidence of
392 a positive relationship between the feeding rate and fecundity. In the present study, although the fecundity
393 estimates (BF and NDO) and lipid classes did not show consistent relationships in ovary and liver, a
394 correlation between BF and muscle TAG and SE-WE was found. The high percentage of BF variability
395 explained by TAG and SE-WE in muscle contrasted with the fact that total lipids in yellowfin tuna
396 muscle exhibited small variations during maturation, and it could be explained as an artefact of the low
397 number of samples used for this analysis. On the other hand, the higher proportion of lipid reserves in the
398 muscle of spawning-capable females may indicate that these fishes were in a better condition which can
399 be translated into a higher fecundity rate as suggested by Schaefer (1998). This strategy is more common
400 for capital breeders species inhabiting temperate water like bluefin tuna (Chapman et al., 2011; Mourente
401 et al., 2002), which have a particularly intense feeding season before the reproductive period. In these
402 species certain somatic tissues, namely, muscle and/or mesenteric perigonadal tissues, exhibit increases in
403 their lipid reserves to be used later during the reproductive cycle. The prediction of fish fecundity through
404 study of maternal condition was described less accurate in income breeder species (Kjesbu, 2009), due to
405 the modulation of reproductive investment is more instantaneously linked to food availability and thus it
406 is more difficult to assess using condition indices. Thus, further research on a greater number of samples
407 and including an analysis of energetic compounds important for reproduction (Kjesbu, 2009) is required
408 to obtain a better overview of factors affecting fecundity in yellowfin tuna.

409

410 *4.3. Lipid class composition regarding school types (FADs vs. free-swimming schools)*

411 Several studies hypothesized that FADs could affect tuna movement and migration, leading them to low-
412 quality habitats, with potentially detrimental effects on individual and population conditions (Hallier and
413 Gaertner, 2008; Marsac et al., 2000). The lipid composition of yellowfin tuna has been observed to be
414 affected by the environmental conditions and feeding quality (Saito et al., 1996). In the present study,
415 female yellowfin tuna caught in free-swimming schools had higher concentrations of total lipids and PL
416 in the gonads and AMPL in the liver than those caught under FADs. Moreover, higher TAG/ST ratios
417 were observed in the gonads of females caught in free-swimming schools. These results might suggest a

418 higher energetic investment in the gonads of females caught in free-swimming schools, and could be
419 interpreted as a sign of these fishes being in a better condition than those caught under FADs. The higher
420 feeding success associated with females in free-swimming schools (Jaquemet et al., 2011) could provide
421 them with larger amounts of energy (e.g., lipids) for reproductive investment. This fact is especially
422 important for species performing income-capital strategy, since the concurrent income energy from
423 feeding is essential for tuning the reproductive effort (McBride et al., 2013). However, the difference in
424 the range of size of sampled individuals between females in free-swimming schools and females under
425 FADs could be a factor affecting the lipid concentration by itself and thus a source of bias. Besides, the
426 absence of a significant relationship between gonad lipids and fecundity suggests that there is no
427 established minimum lipid concentration threshold, indicating that the lower lipid content of FADs
428 females may not jeopardize current reproduction.
429
430 Detailed information on the residence time of the females analysed at FADs is lacking in this study. Data
431 of residence time would allow us to assess whether the suggested detriment of feeding success at FADs
432 (Jaquement et al., 2011) affects lipid acquisition and, hence, individual and population productivity.
433 Therefore, further analyses of fish condition (morphometric and biochemical) based on a better sampling
434 strategy that accounts for school type are required to avoid bias and uncertainty in the sampling, such as
435 geographical and temporal effects, which can affect either the environmental conditions and/or
436 reproductive effort of the species.
437
438 **5. Conclusions**
439 The results provide evidences that the heterogeneity of lipid contents among tissues and the provisioning
440 of lipids for reproduction are related to the reproductive strategy and the role of those tissues during
441 reproduction, as it was observed for other fish species (Johnson, 2009). Yellowfin tuna exhibited a
442 strategy of low energy allocation before reproduction, which was mainly based on lipids stored in the
443 liver. The lack of substantial endogenous energy investment for ovarian development regarding the low
444 lipid content found in tissues could suggest that yellowfin tuna rely, to a large extent, on concurrent
445 energy income from feeding to finance the cost of reproduction. Thus, regarding the combined use of
446 stored lipids and concurrent income energy from prey, together with the reproductive strategy of
447 yellowfin tuna (i.e., asynchronous and indeterminate fecundity), it could be considered that this species is

448 a income-capital breeder. However, further biochemical studies are needed to investigate other important
449 energetic compounds, such as proteins, which would provide a fuller understanding of the energy
450 dynamics during reproduction. In addition, studies of feeding throughout the reproductive cycle are
451 needed to assess variations in the energy supply from prey. Prey availability prior to and during the
452 reproductive cycle will condition the amount of energy stored prior to reproduction and/or the manner in
453 which energy resources are used to offset the cost of reproduction.
454 Finally, although the lipid composition of the main tissues (gonad and liver) involved in reproduction in
455 yellowfin tuna were not strongly correlated with fecundity in this study, food availability is likely to
456 constitute a limiting factor for reproductive potential. Thus, precise characterisation of this relationship of
457 endogenous and income energy supply with fecundity is needed to reveal the impact of abiotic factors
458 such as FADs on the reproductive potential of yellowfin tuna populations.
459

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471

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Table 1. Summary of yellowfin tuna samples collected in the Western Indian Ocean during the three surveys (first survey: from January to March 2009; second survey from June to July 2009; third survey from April to May 2010) by school types (fish aggregating devices [FADs] and free-swimming school [FREE]). Range of fork length (FL) and range of total weight (W) is provided. The collected samples are shown by survey and by ovarian development phases (Dev. Phase): IP = immature phase; DP = developing phase; SCP = spawning-capable phase and RP = regenerating phase.

	2009				2010	
	1st Survey		2nd Survey		3rd Survey	
	FADs	FREE	FADs	FREE	FADs	FREE
n	27	8	17	26	23	11
FL range	50-110	123-153	56-118	122-148	60-120	56-112
W range	2.4-22.4	33.4-63.9	4.2-33.0	31.0-63.0	4.0-31.0	3.7-26.0
Dev. Phase						
IP	8	--	2	--	9	3
DP	16	--	13	12	6	4
SCP	3	8	2	14	--	--
RP	--	--	--	--	8	4

Table 2. Mean (±SE) of condition indices (gonadosomatic index GSI, hepatosomatic index HSI, and condition factor K) and total lipid (TL) concentrations ($\mu g.mg^{-1}ww$) in the gonads, liver and white muscle of female yellowfin tunas collected at different ovarian development phases (Dev. Phase) in the Western Indian Ocean. The number of samples (n) and the fish size range (cm) are specified. Four ovarian development phases were distinguished: IP = immature phase including the primary growth stage (PG); DP = developing phase including the cortical alveoli (CA), primary vitellogenesis (Vtg1) and secondary vitellogenesis (Vtg2); SCP = spawning-capable phase including the tertiary vitellogenesis (Vtg3), germinal vesicle migration (GVM) and hydration (HYD) stages; RP = regenerating phase.

Dev. Phase.	Dev. Stage	n	FL range	GSI	HSI	K	TL Gonads	TL Liver	TL Muscle
IP	PG	22	50-109	0.2±0.1	1.0±0.3	2.0±0.2	6.5±0.1	20.8±0.1	5.9±0.1
DP	CA	35	50-145	0.3±0.1	0.8±0.3	1.9±0.2	4.8±0.1	16.2±0.1	4.9±0.1
	Vtg1	8	76-148	0.6±0.3	0.7±0.2	1.9±0.3	7.3±0.2	14.2±0.2	5.3±0.2
	Vtg2	8	69-146	0.7±0.3	0.7±0.1	2.0±0.3	13.2±0.2	14.3±0.2	4.4±0.2
SCP	Vtg3	18	88-145	1.1±0.5	0.8±0.2	2.0±0.3	26.7±0.2	13.7±0.2	5.3±0.2
	GVM	5	123-146	1.7±0.4	1.0±0.1	1.7±0.0	25.8±0.4	10.3±0.4	3.4±0.4
	HYD	4	130-153	1.4±0.6	1.1±0.1	1.8±0.0	11.6±0.4	19.8±0.4	2.9±0.5
RP		12	102-120	0.3±0.1	0.7±0.1	1.9±0.1	5.2±0.2	17.2±0.2	4.4±0.2

Table 3. Summary of the mixed ANCOVA model (*P*-values) on the effects of the female yellowfin maturity (i.e., ovarian developmental phases and stages) on the concentrations of total lipids and lipid classes in three tissues (gonads, liver and muscle). Results of the interactions between the tissues, condition indices (GSI = gonadosomatic index; HIS = hepatosomatic index; K = condition factor) and fork length (FL) are provided. TAG = triacylglycerols; SE-WE = sterol- and wax-esters; PL = phospholipids; ST = sterols; AMPL = acetone mobile polar lipids; KET = ketones.

	TAG	SEWE	PL	ST	AMPL	KET	Total Lipids
Maturity	**0.029**	**0.004**	**0.038**	0.074	0.547	0.401	**0.009**
Tissue	**0.004**	0.360	0.616	**0.023**	**0.015**	0.746	**0.023**
FL*Tissue	**<0.001**	0.514	0.199	**0.003**	**0.002**	0.058	**<0.001**
GSI*Tissue	0.801	0.292	0.348	0.564	0.863	0.829	0.997
HSI*Tissue	**<0.001**	0.150	0.559	**0.009**	0.659	**0.002**	**0.001**
K*Tissue	0.066	0.073	0.755	0.325	0.055	0.976	0.165
Maturity*Tissue	**0.002**	**<0.001**	**<0.001**	**0.014**	**0.016**	0.360	**<0.001**

Table 4. Summary of the significant results of the stepwise regression model selection between (i) the muscle lipid composition and the batch fecundity (BF) and the gonad lipid composition and number of developing oocyte (NDO) of yellowfin tuna, (ii) fish condition and batch fecundity (BF). GSI = gonadosomatic index; TAG = triacylglycerols; SE-WE = sterol- and wax-esters; ST = sterols.

Lipid composition	Step	Variable	r^2	*F*-value	*P*-value
BF	1	TAG	0.644	12.67	0.009
	2	SEWE	0.217	9.37	0.022
NDO	1	ST	0.410	11.10	0.004
Condition Indices	Step	Variable	r^2	F-value	P-value
BF	1	GSI	0.755	18.47	0.005

Table 5. Summary of yellowfin tuna females at developing and spawning-capable phases included in the analysis of the effects of the school-type on the concentrations of total lipids and lipid classes in three tissues (gonads, liver and muscle). The number of samples (n), the fork length range (FL; cm), and weight range (W; kg) are specified. Mean (±SE) of condition indices (gonadosomatic index GSI, hepatosomatic index HSI, and condition factor K) and total lipid (TL) concentrations ($\mu g.mg^{-1}ww$) in the gonads, liver and white muscle are provided.

School-type	n	FL range	W range	GSI	HSI	K	TL Gonads	TL Liver	TL Muscle
FADs	14	69-117	8.5-29	0.6±0.4	0.9±0.3	2.1±0.2	12.6±0.2	12.7±0.2	5.3±0.2
FREE	25	122-148	31-63	1.2±0.5	0.8±0.2	1.9±0.3	18.9±0.1	17.4±0.1	3.7±0.1

Table 6. Summary of the mixed ANCOVA model (*P*-values) on the effects of the school-type on the concentrations of total lipids and lipid classes in three tissues (gonads, liver and muscle) of the yellowfin tuna. Results of the interactions between the tissue and the school-type (FADs and free-swimming school) are provided. TAG = triacylglycerols; SE-WE = sterol- and wax-esters; PL = phospholipids; ST = sterols; AMPL = acetone mobile polar lipids; KET = ketones.

	TAG	SEWE	PL	ST	AMPL	KET	Total Lipids
School-type	0.434	0.408	**0.033**	0.683	0.198	0.743	0.233
Tissue	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
School-type*Tissue	0.101	0.373	**0.002**	0.719	**0.038**	0.487	**0.016**

Figure caption

Fig. 1 The locations at which female yellowfin tuna (*Thunnus albacares*) were sampled in the Western Indian Ocean during 2009 and 2010 by school type: free-swimming schools (+) and fish aggregating devices (FADs) (●).

Fig. 2 Significant linear regressions ($p<0.05$) derived from the mixed ANCOVA model between yellowfin tuna fork length (FL, cm) and (A) gonad triacylglycerol (TAG) concentration, (B) gonad triacylglycerol:sterol (TAG/ST) ratio and (C) muscle total lipid (cTotal) concentration, as well as between the hepatosomatic index (HSI) and liver TAG/ST ratio (D).

Fig. 3 Concentrations ($\mu g.mg^{-1}$ ww) of (A) triacylglycerols (TAG), (B) sterol- and wax-esters (SE-WE), (C) phospholipids (PL), (D) sterols (ST), (E) acetone mobile polar lipids (AMPL) and (F) total lipids in the gonads (●), liver (●) and white muscle (▼)of female yellowfin tuna from the Western Indian Ocean as a function of maturity (i.e., ovarian development phases and stages). The letters show the significant differences between the different stage of oocyte development and the three tissues.

Fig. 4 Results of the significant interaction between school type and tissue (gonads, liver and muscle) for yellowfin tuna described by the mixed ANCOVA model for (A) phospholipids (PL), (B) total lipids and (C) acetone mobile polar lipids (AMPL). The letters show the significant differences between females caught at FADs and females caught in free-swimming schools by tissue.

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820 **Fig.1**

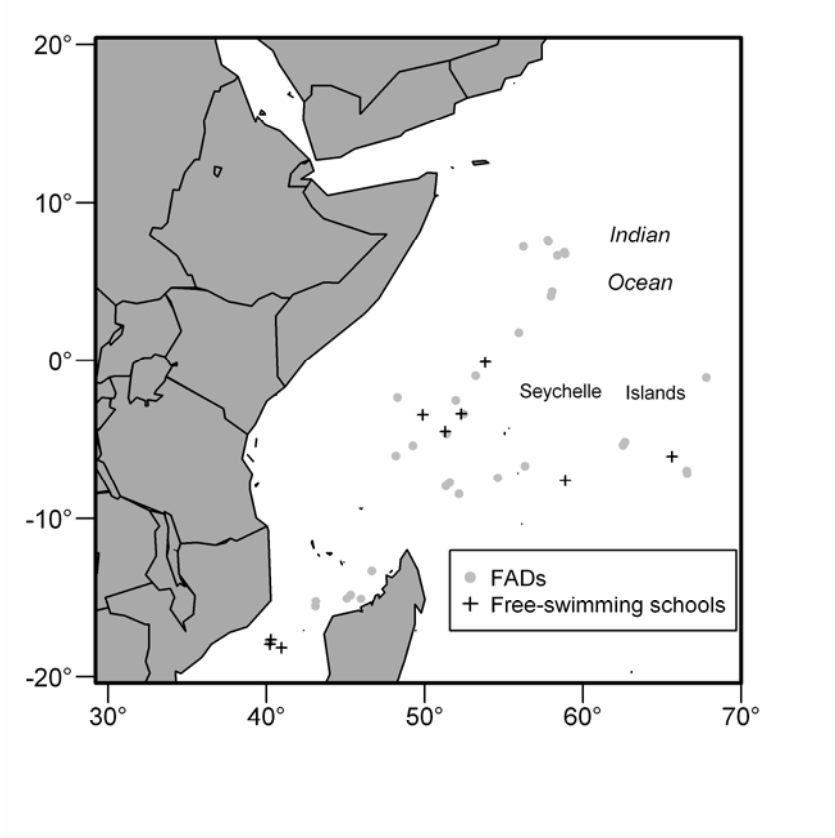

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Fig.2

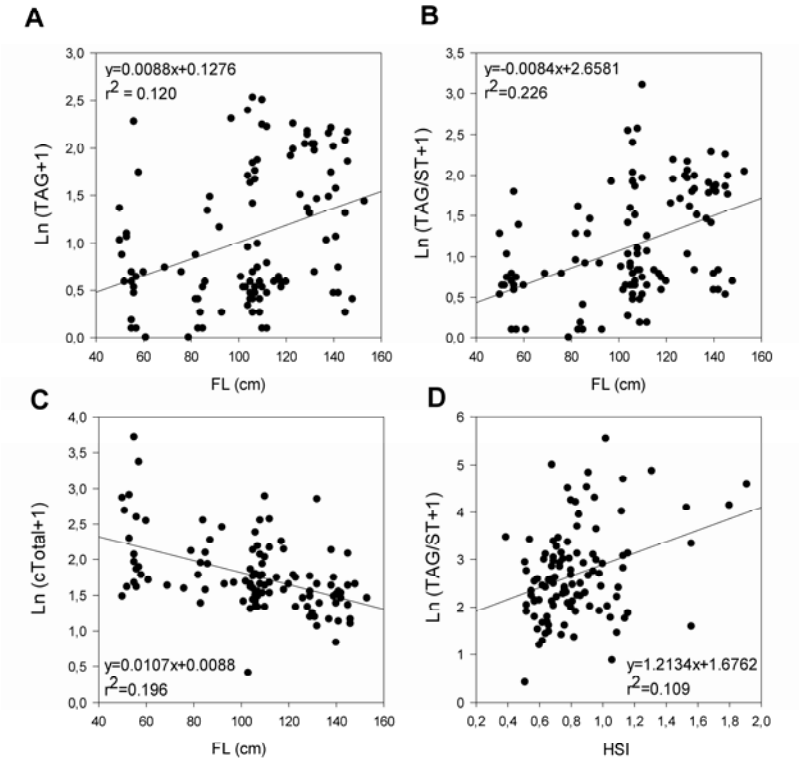
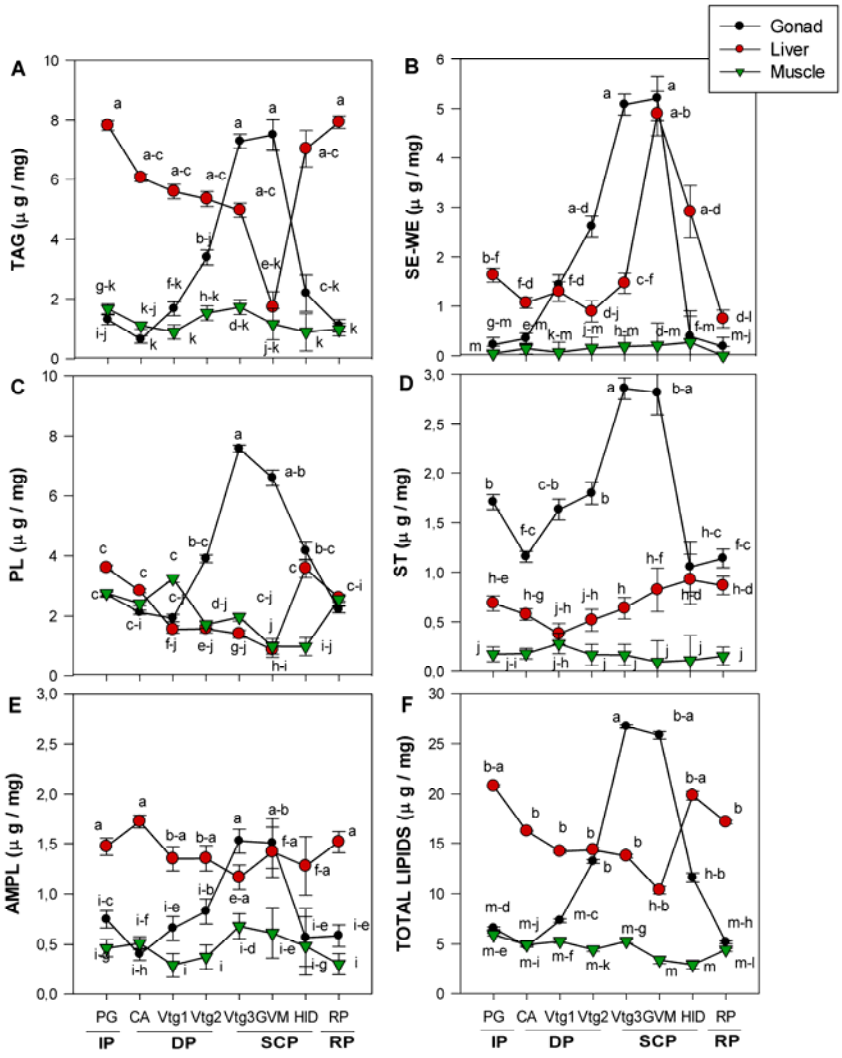
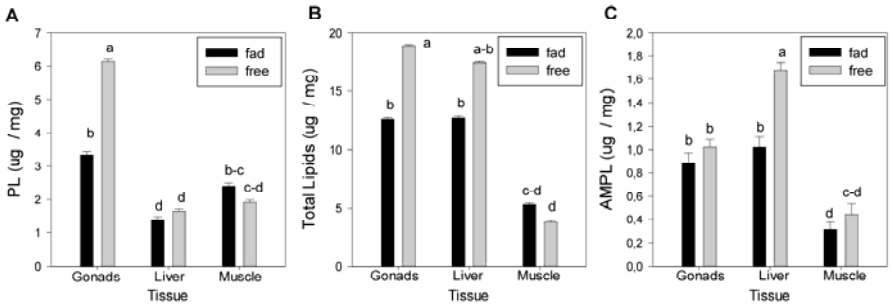

A
y=0.0088x+0.1276
r2 = 0.120
Ln (TAG+1)
FL (cm)
B
y=-0.0084x+2.6581
r2=0.226
Ln (TAG/ST+1)
FL (cm)
C
y=0.0107x+0.0088
r2=0.196
Ln (cTotal+1)
FL (cm)
D
y=1.2134x+1.6762
r2=0.109
Ln (TAG/ST+1)
HSI

Fig.3


Gonad
Liver
Muscle
A
TAG (µ g / mg)
B
SE-WE (µ g / mg)
C
PL (µ g / mg)
D
ST (µ g / mg)
E
AMPL (µ g / mg)
F
TOTAL LIPIDS (µ g / mg)
PG CA Vtg1 Vtg2 Vtg3 GVM HID RP
IP DP SCP RP

866

867 **Fig.4**



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