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Additional Information

Relationship between sperm quality parameters and the fatty acid composition of the muscle, liver and testis of European eel

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35 **Abstract**
36 This study looks at the correlations of fatty acids has on different tissues in the
37 European eel (*Anguilla anguilla*, L.) during hormonally-induced sexual maturation,
38 with different sperm quality parameters. In order to evaluate the different dynamics of
39 the use of fatty acids, a categorization of the results from each sperm quality parameter
40 (volume, concentration, motility and velocity) was performed.
41 Low and moderate correlations were observed between muscle tissue and some sperm
42 quality parameters but no high correlations were found. Eicosapentaenoic acid (20:5n3,
43 EPA) in the liver seems to have a role in determining the volume of sperm produced.
44 This can be explained by the fact that EPA is a major requirement in the early phases of
45 sperm production (probably as a component of the spermatozoa membrane). In addition,
46 the levels of α-linolenic acid (18:3-n3, ALA) and linoleic acid (18:2-n6, LA) in the liver
47 decreased when sperm motility increased. In all the tissues, a negative correlation was
48 observed between arachidonic acid (20:4n-6, ARA) and the different sperm velocity
49 parameters. The fact that an increase in the consumption of ARA coincides with an
50 increase in the speed of spermatozoa, highlights the important role that this fatty acid
51 plays not only in sperm production, but also in sperm velocity.
52 All this information could prove useful in the development of suitable broodstock diets
53 to improve sperm quality and subsequently, the larval development of this species.
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57 **Keywords:**
58 Sperm motility; Sperm Velocity; PUFA; Broodstock diet; Spermatogenesis
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69 **1. Introduction**

70 Over the past 25 years European eel populations have been declining. Several factors
71 such as infections, pollution, overfishing and habitat destruction, mean that the stock is
72 now considered outside of safe biological limits and immediate protection measures
73 have been recommended (Van den Thillart and Dufour, 2009; ICES, 2011).It is known
74 that in the autumn eels begin their maturation to the silver eel stage, when they descend
75 from the rivers and migrate to the sea. Spawning occurs between April and June,
76 between 200-600 m, in the Sargasso Sea (Aarestrup et al., 2009), although many details
77 of the migration still remain unknown.

78 A key factor in the success of eel reproduction in captivity is good quality gametes
79 (both eggs and sperm), and therefore it is important to consider different hormonal
80 induction treatments. The sexual maturation of males can be induced by using long-term
81 hormonal treatments (Ohta et al., 1997; Pérez et al., 2000; Asturiano et al., 2005; Huang
82 et al., 2009; Gallego et al., 2012). The effect of different hormonal treatments and
83 environmental parameters on gamete quality has been studied in both Japanese and
84 European eels (Miura et al., 1991; Asturiano et al., 2005; Gallego et al., 2012; Mazzeo
85 et al., 2012) but the effect of broodstock feeding on gamete quality has been
86 investigated in female eels (Furuita et al., 2006, 2007; Ozaki et al., 2008; Oku et al.,
87 2009; Støttrup et al., 2012), not in males.

88 An assessment of sperm quality is important in order to ensure the success of the
89 reproduction process. It is clear that broodstock nutritional requirements have to be met
90 in order to achieve reproductive performance, and several studies indicate that the
91 composition of dietary lipids affects male reproductive performance in different
92 teleosts, including the European sea bass (*Dicentrarchus labrax*; (Bell et al., 1996;
93 Asturiano et al., 2001), Eurasian perch (*Perca fluviatilis*; (Henrotte et al., 2010)),
94 rainbow trout (*Oncorhynchus mykiss*; (Labbe et al., 1995;Pustowka et al., 2000)), Indian
95 major carp (*Catla catla*; (Nandi et al., 2007)) and African catfish (*Clarias gariepinus*;
96 (Nyina-Wamwiza et al., 2012))

97 The effect of male broodstock feed on sperm quality has not been studied in either
98 European eel or Japanese eel. Mazzeo et al. (2010) studied the changes in fat and fatty
99 acid levels in the muscle, liver and testis of European eel throughout spermatogenesis.
100 More recently, the variations in the levels of fatty acids in different tissues of males
101 undergoing hormonal induction at different thermal regimes were studied (Baeza et al.,
102 2014).

103 Little is known about the effect of fatty acids on the sperm quality of eels. Although eels
104 cease to feed from the onset of sexual maturation (Tesch, 2003), the body composition
105 at the time of sexual maturation is fundamental, and developing suitable diets appears to
106 be essential for reproductive success. The aim of this research was to clarify the
107 variations in the fatty acid composition of different tissues and to determine whether
108 there is any link with the changes in sperm quality parameters. The knowledge
109 generated will be implemented in broodstock diets to potentially improve sperm quality.
110

111 **2. Material and methods**

112 **2.1 Fish maintenance and hormonal treatment**

113 Three hundred and seventeen male eels (mean body weight 100 ± 2 g) were moved
114 from the Valenciana de Acuicultura, S.A. fish farm (Puzol, Valencia; East coast of
115 Spain) to the facilities of the Universitat Politècnica de València (Spain). The animals
116 were placed in six 200 L aquaria equipped with separated recirculation systems,
117 thermostats and coolers and covered to maintain constant shade. The fish were
118 gradually acclimatized to sea water (salinity 37 ± 0.3 g L^{-1}) over the course of a week
119 and were fasted during both the acclimatization and the experimental periods.
120 The fish underwent three thermal regimes: T10, (10 ºC for the first 6 weeks, 15 ºC for
121 the next 3 weeks and 20 ºC for the last 6 weeks); T15, (15 ºC for the first 6 weeks and
122 20 ºC for the last 9 weeks); and T20, (20 ºC throughout the whole experimental period).
123 For 13 weeks, the males were hormonally treated to induce maturation and spermiation
124 through weekly intraperitoneal injections of human chorionic gonadotropin (hCG; 1.5
125 IU g^{-1} fish; Argent Chemical Laboratories. USA) as previously described by Pérez et al.
126 (2000).
127 Different spermiation patterns were observed depending on the initial water
128 temperature. At the sampling time, all the fish were at 20 ºC because this is the water
129 temperature needed in order for the eels to produce sperm. The samples used to
130 determine the relationship between the fatty acid levels and the sperm quality
131 parameters were collected once sperm production had been achieved, independently of
132 the initial temperature. Samples from T20 fish were collected at 5th week, T15 samples
133 were collected at 7th and T10 samples were collected at 10th week.
134

135 **2.2 Samplings and sperm collection**

136 Between 5 and 8 fish per thermal regime were sacrificed each week by decapitation,

137 after having previously been anesthetized with benzocaine (60 ppm). Only spermiating
138 males were sampled and fatty acids from muscle, liver and testis were correlated with
139 the sperm parameters. A different number of samples was obtained from each group,
140 depending on the length of the spermiation period: in the case of T10, a total of 12 fish
141 were sampled from the 10th week; in the case of T15, a total of 32 fish were sampled
142 from the 7th week; T20, a total of 47 males were sampled from the 5th week.
143 The sperm was collected by applying gentle abdominal pressure to previously
144 anesthetized males after cleaning the genital area with distilled water to avoid
145 contamination with faeces, urine or seawater. A small aquarium air pump was modified
146 to obtain a vacuum suction force, and the sperm was collected in a tube. Sperm samples
147 were collected 24 h after the administration of the hormone because previous studies
148 (Pérez et al., 2000) have demonstrated that this is when sperm quality is higher.
149 Samples from the muscle, liver and testis were collected. The muscle was crushed in a
150 meat grinder and homogenized before storage. All the samples were stored at -80 ºC
151 until lipid extraction and fatty acid quantification.
152

153 **2.3 Lipid and fatty acid analysis of tissues**

154 Total lipids from muscle were extracted in Soxtec extraction unit (1043, Tecator). The
155 total lipids from the testis and the liver were extracted using a modified version of the
156 Folch method (Folch et al., 1956). A direct method of FAME synthesis was performed
157 according to O´Fallon et al. (2007). Fatty acid quantification was carried out by gas
158 chromatography. All the methodologies used were carried out and described deeply in
159 Baeza et al. (2014).
160

161 **2.4 Determination of sperm concentration and volume**

162 Dilutions to measure the sperm concentration was did according Asturiano et al. (2004).
163 Sperm volume (mL) and concentration were carried out following the methodologies
164 specified in Gallego et al. (2012).
165

166 **2.5 Evaluation of sperm motility and velocity parameters**

167 A standardized methodology was used for the motility analysis (MOT) (Gallego et al.,
168 2013). Cells were considered to be “Progressive motile cells” when they swim forward
169 in 80% of a straight Line (P-MOT). Different velocity parameters were assessed,
170 including: curvilinear velocity (VCL, μm/s), defined as the time/average velocity of a

171 sperm head along its actual curvilinear trajectory; straight line velocity (VSL, µm/s),
172 defined as the time/average velocity of a sperm head along the straight line between its
173 first detected position and its last position; average path velocity (VAP, µm/s), defined
174 as the time/average of a sperm head along its spatial average trajectory. All motility and
175 velocity analyses were performed by Gallego et al. (2012).
176

177 **2.6 Parameter categorization and statistical analysis**

178 The fatty acids were quantified in each tissue (muscle, liver and testis) to estimate the
179 possible correlation with the sperm quality parameters. First, linear correlations between
180 sperm quality parameters and fatty acids were performed for each tissue, with the data
181 obtained from the different thermal regimes considered separately. No linear
182 correlations were however appreciated. Then, nonlinear regressions (which could be
183 considered more powerful in the evaluation of these kinds of parameters) were carried
184 out. Several significant but low correlations were found when the whole data range from
185 each thermal regime was considered. This could be due to fact that fatty acids have
186 different roles throughout the final sperm maturation process. We therefore decided to
187 analyze the data in an alternative way. The results of each sperm quality parameter were
188 categorized in order to better analyze the data and try to find all the meaning from the
189 results. The established parameter ranges and categories are shown in table 1.
190 The number of samples from each category was very low when the thermal treatments
191 (T10, T15 and T20) were considered separately, but all the samples were obtained from
192 fish producing sperm once over a threshold temperature of 20 ºC. The absence of
193 statistical differences was checked by one way ANOVA comparing the means of the
194 fatty acids (the thermal regimes were considered separately) in relation to each sperm
195 quality parameter categorized. Prior to this, data normality had been checked using the
196 asymmetry standard coefficient and Curtosis coefficient. For example, P-value results
197 of categorized VCL showed no significant differences in any tissue (Supplementary
198 material Tables 1-3). So, in order to get a higher number of samples all the data were
199 considered together and correlations were made independently of the thermal regime.
200 Pearson´s correlation, coefficient of determination and linear regression analyses
201 ($P<0.05$) were used to determine the relationship between each fatty acid and the
202 different categorized parameters. All the statistical analyses were performed using the
203 statistical package SPSS version 19.0 for Windows software (SPSS Inc., Chicago, IL,
204 USA).

205 **3. Results**

206 **3.1 Muscle**

207 The correlations found between the sperm quality parameters and fatty acids in the
208 muscle are shown in Table 2. The low sperm concentration categories (CON1 and
209 CON2; 0-10x10^9 cells mL^{-1}) showed significant moderate negative correlations
210 (P<0.05) with several fatty acids: palmitic acid (16:0), stearic acid (18:0)and EPA with
211 CON1, and oleic acid (18:1-n9) with CON2.
212 No significant correlation between motility and any fatty acid in particular was found,
213 but a positive correlation between n-3/n-6 ratio and MOT1 was registered, with a
214 greater proportion of n-3 series fatty acids present when motility was between 0 and
215 25%. The progressive motile spermatozoa (P-MOT), specifically P-MOT2, were
216 negatively correlated (P<0.05) with monounsaturated fatty acids (MUFA) including
217 palmitoleic acid (16:1) and vaccenic acid (18:1-n7), and consequently with total MUFA
218 (P<0.05). Regarding the sperm velocity parameters, only VSL3 and VAP3 showed
219 significant correlations (P<0.05) with several fatty acids in the muscle. VSL3 was
220 negatively correlated with DHA, ARA and their precursors ALA and LA. On the other
221 hand, VAP3 showed significant positive correlations (being P<0.05 in all the cases)
222 with the following fatty acids: myristic acid (14:0), total SFA and n-9 fatty acids such as
223 eicosenoic acid (20:1-n9) and erucic acid (22:1-n9).
224

225 **3.2 Liver**

226 The correlations found between the sperm quality parameters and liver fatty acids are
227 shown in Table 3. All the sperm volume categories showed significant correlations with
228 different fatty acids, in most cases, from the n-3 series. A negative correlation at P<0.01
229 level between VOL2 and EPA (Fig. 1A) was found.
230 A moderate negative correlation was registered between sperm motility in the different
231 categories (P<0.05) and several fatty acids: 18:0, EPA, total PUFA and total n-3 series
232 fatty acids with MOT2, and 16:0, 18:1-n9, total SFA and total MUFA with MOT3.
233 In terms of progressive motile spermatozoa, Figure 2 (A and B) shows the high negative
234 correlations (P<0.01) between P-MOT4 and ALA and LA fatty acids, respectively.
235 Regarding the sperm velocity parameters, only VAP (but not VCL and VSL) was
236 positively correlated with some liver fatty acids: ALA, LA, DHA and total n-6 series
237 fatty acids. On the other hand, a positive correlation was registered between VAP3 and
238 14:0 and a negative correlation with ARA in the liver (P<0.05).

239 **3.3 Testis**

240 The correlations between the sperm quality parameters and testis fatty acids are shown
241 in Table 4. The ARA/EPA ratio was negatively correlated with concentration when this
242 was higher than $15x10^9$ cells mL^{-1}.
243 With regards to P-MOT, 22:1-n9 was positively correlated in the testis when the
244 percentage of progressive spermatozoa was 5-15% (P-MOT2, $P<0.05$). P-MOT3
245 showed significant negative correlations ($P<0.05$) with the following fatty acids: 18:0
246 and n3-series fatty acids such as EPA, 22:5-n3 and DHA.
247 When considering the sperm velocity parameters, many testis fatty acids were positively
248 correlated with the highest curvilinear velocities (VCL4, >130 µm/s): 14:0, 16:0, 18:1-
249 n9, 18:1-n7, ALA, LA, total SFA (with $P<0.05$) and 16:1, 20:1-n9, 22:1-n9, and total
250 MUFA (with $P<0.01$). There was also a significant high negative correlation at $P<0.01$
251 level between VCL4 and the ARA and ARA/EPA ratio in the testis (Fig. 3), suggesting
252 a reduction of ARA at the end of the sperm maturation process.
253 In terms of the VSL values, 18:0 ($P<0.01$), EPA, total PUFA and total n-3 series fatty
254 acids ($P<0.05$), were negatively correlated with VSL3.
255 A relationship between VAP and fatty acids in the testis, with positive and negative
256 correlations was found. A significant positive correlation was registered between VAP3
257 and EPA and a negative correlation between VAP3 and the EPA/DHA ratio ($P<0.05$).
258 Moderate negative correlations ($P<0.01$) were also found between VAP4 and the ARA
259 and ARA/EPA ratio.
260

261 **4. Discussion**

262 We used data from Gallego et al. (2012), who registered sperm volumes similar to those
263 described by other authors (1–4 mL 100 g $fish^{-1}$; (Pérez et al., 2000; Asturiano et al.,
264 2005) and, an increasing trend in sperm volume over the weeks of spermiation.
265 Regarding the correlations found between sperm volume and the fatty acids present in
266 the different tissues, significant correlations were found with the liver fatty acids. Levels
267 of EPA, n-3 series fatty acids and total MUFA decreased in the liver when sperm
268 volume was between 0-3 mL. This decrease in EPA meant that the ARA/EPA ratio was
269 positively correlated with sperm volume. Our hypothesis suggests that, when the
270 volume of sperm being produced is low, EPA could be being synthesized in the liver
271 (negative correlation found in the liver with sperm 0.5-1 mL volumes) and being sent to
272 the testis, which require EPA for the production of the sperm cell membranes (Lenzi et

273 al., 2000). Figure 4A shows the main fatty acid mobilization from the liver to the gonad
274 during eel spermatogenesis (described by Baeza et al., 2014). Figure 4B shows (in
275 green) the correlations between EPA from the liver and sperm volume.
276 Pérez et al. (2000), in their analysis of European eel sperm fatty acids, found significant
277 negative linear correlations between sperm volume and total n-3 fatty acids, EPA and
278 DHA. Our results also show negative correlations between sperm volume with different
279 n-3 series fatty acids but in our case from the liver, supporting the important role of this
280 tissue (especially when eels produce sperm) highlighted by Baeza et al. (2014). Similar
281 results were found by Pérez et al. (2000) in the sperm, suggesting a connection. In our
282 opinion, the decrease of n3 fatty acids in liver coinciding with the sperm volume
283 increase could be due to their mobilization to the gonad, where spermatozoa use them,
284 with the consequent reduction of n3 fatty acids also in sperm. In fish species has
285 demonstrated the influence of dietary fatty acids on sperm concentration. Nandi et al.
286 (2007) showed that spermatozoa concentration and spermatocrit in Indian major carp
287 (*Catla catla*) were significantly higher in fish fed PUFA enriched test diets than fish fed
288 control diet. Furthermore, fatty acid supplementation in male European sea bass induced
289 a longer spermiation period and higher milt spermatozoa concentrations (Asturiano et
290 al., 2001). Recently, a higher sperm concentration was found in rats fed a diet with a
291 high n-3/-6 fatty acids ratio (Yan et al., 2013). Moreover, in humans, sperm
292 concentration has been positively correlated with DHA levels (Nissen et al., 1983). All
293 of these results highlight the influence of fatty acids in relation to sperm concentration
294 and, in the present study, although several negative correlations between fatty acids and
295 different concentrations were found especially in muscle, we only can propose a
296 hypothesis. The decrease found in these fatty acids in the muscle can be explained by
297 their mobilization to other tissues, where local consumption might occur, explaining
298 why no increases were registered in the other tissues. In the testis, when the highest
299 concentrations were registered, there was a negative correlation with ARA/EPA, due to
300 an increase in ARA. Figure 4B shows (in orange) the most important correlations
301 between the fatty acids and the sperm concentration in the three tissues. Beirão et al.
302 (2012) studied the lipid content of sperm flagella and head membrane of gilthead
303 seabream (*Sparus aurata*) and suggest that fatty acid composition differs depending on
304 their function and their effect on sperm motility and viability. Vassallo-Agius et al.
305 (2001) showed that motility was lower in rainbow trout (*Oncorhynchus mykiss*) fed an
306 n-3 essential fatty acid deficient diet compared to a control group fed a commercial diet,

307 highlighting the importance of PUFA in sperm motility, just as in humans (Lenzi et al.,
308 2000). Recently, Butts et al. (2011) in studies of Atlantic cod (*Gadus morhua*)
309 suggested that differences observed in fatty acid composition between wild and
310 cultivated cod sperm derived from their diets and influenced sperm activity. In the
311 present study, several correlations were found between MOT and P-MOT and fatty
312 acids in all the tissues analysed, highlighting again, as previously reported in other
313 publications, the relationship between fatty acids and sperm motility. This suggests that
314 these fatty acids are probably used as an energy source to increase sperm energy for
315 motility requirements. Recently, Mehdinejad et al. (2013) highlighted the importance of
316 fatty acids for sperm movement in Iranian sturgeon (*Acipenser persicus*). Furthermore,
317 there was a high negative correlation between LA and ALA in the liver and progressive
318 motile cells (Fig. 2). An explanation for all of the obtained results could be that the liver
319 synthesizes PUFA from their precursors (LA and ALA, also PUFAs) and sends them to
320 the testis, where they are used to increase sperm motility. The importance of fatty acids,
321 especially PUFA, in sperm motility has been demonstrated in other animal species. For
322 example, in boars fed a diet supplemented with shark oil, rich in PUFA, an
323 improvement in sperm motility was found (Mitre et al., 2004). Again in boars, DHA
324 and n-3 series fatty acids in sperm were positively correlated with motility (Am-in et al.,
325 2011). Furthermore, in rats sperm motility was found to be positively correlated with
326 the n-3/n-6 fatty acids ratio in the diet (Yan et al., 2013). Additionally, in humans sperm
327 motility was negatively correlated to the seminal plasma concentration of n-6 series
328 fatty acids (Safarinejad et al., 2010) and also in humans, motility has been positively
329 correlated with the DHA levels in sperm (Nissen et al., 1983) although DHA
330 supplementation does not affect human sperm motility (Conquer et al., 2000). All the
331 studies listed above, have highlighted the main role of PUFA on sperm motility, and
332 concur with the results of the present study into eel, where correlations between motile
333 cells with ALA and LA (both PUFAs) from liver were found. Figure 4B summarizes (in
334 blue) the most important correlations between fatty acids from the liver and the muscle
335 and sperm motility.
336 Sperm speed improved in African catfish (*Clarias gariepinus*) fed a diet in which
337 fishmeal was completely substituted by agricultural products and consequently had high
338 levels of n-6 series fatty acids (Nyina-Wamwiza et al., 2012). Martínez-Páramo et al.
339 (2012) in a study where they evaluated the correlation between sperm lipid peroxidation
340 and the sperm quality of precocious European sea bass (*D. labrax*), found a positive

341 correlation between VSL and the amount of ARA in the sperm, as well as a negative
342 correlation with the DHA/EPA ratio.
343 The principal results from the present study have shown a negative correlation between
344 ARA in all the tissues and the different categorized velocities and in particular, a high
345 negative correlation in the testis between ARA and the highest VCL (Fig. 3). Therefore,
346 this may indicate that ARA is metabolized to form prostaglandins which are involved in
347 steroid production (Wade and Van der Kraak, 1993), which may help increase the speed
348 of the spermatozoa, thus highlighting the importance of this fatty acid. Eels do not feed
349 during the maturation and spermiation period and, their energy reserves are consumed
350 to maintain their metabolism and also to carry out several processes as gonad formation
351 (Baeza et al., 2014) or, as in the present study have been described, energy from fatty
352 acids could be used to increase the motility and velocity of sperm. Recently, tests were
353 carried out on Senegalese sole (*Solea senegalensis*), using diets with different contents
354 of ARA along the reproductive cycle, and it was found that the presence of ARA in
355 tissues differs depending on the sex (Norambuena et al., 2012). In the present study,
356 negative and positive correlations were found (but lower than in the case of ARA)
357 between EPA in the testis and the highest registered velocities. Recently, EPA seems to
358 have a modulatory effect on the synthesis of androgens in eels with mature sperm
359 (Baeza et al., 2015) so, both fatty acids (ARA and EPA) play important roles in male
360 reproduction. ARA has been shown to be the main precursor for the production of
361 series-2 prostaglandins, whereas it has been reported that EPA functions as an inhibitory
362 regulator (Asturiano et al., 2000; Sargent et al., 2002) and in our study, both ARA and
363 EPA appear to have an important function with regards to spermatozoa velocity. Baeza
364 et al. (2014) also highlighted the importance of these fatty acids (ARA and EPA) in
365 male European eel reproduction, stating that EPA seems to be mobilized from the liver,
366 where it has previously been synthesized. Figure 4B shows (in red) the most important
367 correlations between ARA from the three tissues and sperm velocity.
368 Another important result from the present study is that, in different tissues (muscle and
369 testis), a positive correlation between several n-9 series fatty acids (20:1-n9 and 22:1-
370 n9) and higher sperm velocities was found. The eels used in this study came from a fish
371 farm and these fatty acids are not usually present when they feed in the wild. Positive
372 correlations were found between these n-9 series fatty acids and velocity parameters,
373 which could prove important in the design of broodstock diets for male European eel
374 and in the improvement of sperm quality.

375 **5. Conclusions**

376 Overall our results suggest that, in the European eel, fatty acids, and in particular ARA,
377 EPA, ALA and LA are linked to sperm quality parameters. All of this information,
378 together with the conclusions made by Baeza et al. (2014, 2015), could prove useful in
379 the development of enriched diets that may improve sperm quality, which in turn, could
380 have an impact on the reproductive abilities of European eel males, thus improving
381 fertilization success and embryo development. With the importance of PUFA in mind
382 we propose further research aimed at improving the reproductive performance of eels by
383 manipulating dietary requirements. The first step for further investigations might be to
384 try to find the optimum inclusion levels of these fatty acids for commercial diets.

385

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397

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547 **Table legend**

548

549 **Table 1.** Categorization of sperm parameters.

550

551 **Table 2.** Correlations between muscle fatty acids and sperm quality parameters are
552 shown. Asterisks indicate significant correlations between parameters and fatty acids (*,
553 p-*value* < 0.05; **, p-*value* < 0.01). VOL: Sperm volume; DEN: Sperm concentration;
554 MOT: Sperm motility; PMOT: Progressive motile sperm; VSL: Straight line velocity of
555 sperm; VAP: Average path velocity of sperm.

556

557 **Table 3.** Correlations between liver fatty acids and sperm quality parameters are shown.
558 Asterisks indicate significant correlations between parameters and fatty acids (*, p-
559 *value* < 0.05; **, p-*value* < 0.01). VOL: Sperm volume; DEN: Sperm concentration;
560 MOT: Sperm motility; PMOT: Progressive motile sperm; VAP: Average path velocity
561 of sperm.

562

563 **Table 4.** Correlations between testis fatty acids and sperm quality parameters are
564 shown. Asterisks indicate significant correlations between parameters and fatty acids (*,
565 p-*value* < 0.05; **, p-*value* < 0.01). DEN: Sperm concentration; MOT: Sperm motility;
566 P-MOT: Progressive motile sperm; VCL: Curvilinear velocity of sperm; VSL: Straight
567 line velocity of sperm; VAP: average path velocity of sperm.

568 **Figure legend**

569

570 **Figure 1.** Relationship between VOL2 (Sperm volume) and EPA in the liver (n=15).
571 Linear regression equation was calculated for each parameter.

572

573 **Figure 2.** Relationship between: A) P-MOT4 (Progressive motile sperm) and 18:3-n3
574 and; B) P-MOT4 (Progressive motile sperm) and 18:2-n6 in the liver (n=18). Linear
575 regression equations were calculated for each parameter.

576

577 **Figure 3.** Relationship between: A) VCL4 (Curvilinear velocity of sperm) and ARA
578 and; B) VCL4 (Curvilinear velocity of sperm) and ARA/EPA in the testis (n=33).
579 Linear regression equation was calculated for each parameter.

580

581 **Figure 4.** A) Fatty acid dynamics during eel spermatogenesis. Fatty acid content did not
582 change in muscle. Liver highlighted as the main site of synthesis and in gonad EPA,
583 ARA and DHA remained constant while the rest decrease. B) Summary of the
584 correlations between the most important fatty acids and sperm quality parameters in
585 different tissues. The three main conclusions were: 1- EPA in liver decreased when
586 sperm volumes increased; 2- ALA and LA (PUFA precursors) decreased in liver when
587 motility increased; 3- ARA levels decreased in all tissues when sperm velocity
588 increased.

589 **Table 1**

590

Parameter	Category	Range
Volume (mL 100 g^{-1} fish)	VOL1	0-0.5
	VOL2	0.5-1
	VOL3	1-3
	VOL4	>3
Concentration (10^9 cells mL^{-1})	CON1	0-5
	CON2	5-10
	CON3	10-15
	CON4	>15
Total Motility (% motile cells)	MOT1	0-25
	MOT2	25-50
	MOT3	>50
Progressive Motility (% progressive motile cells)	P-MOT1	0-5
	P-MOT2	5-15
	P-MOT3	15-25
	P-MOT4	>25
Curvilinear Velocity (µm/s)	VCL1	0-50
	VCL2	50-100
	VCL3	100-130
	VCL4	>130
Straight Line Velocity (µm/s)	VSL1	0-30
	VSL2	30-50
	VSL3	50-80
	VSL4	>80
Average Path Velocity (µm/s)	VAP1	0-30
	VAP2	30-50
	VAP3	50-80
	VAP4	>80

591 **Table 2**

592

Fatty acid	VOL3 n=29	CON1 n=25	CON2 n=28	MOT1 n=31	P-MOT2 n=16	VSL3 n=30	VAP3 n=28
14:0							0.410*
16:0		-0.498*					
16:1					-0.559*		
18:0	-0.388*	-0.462*					
18:1-n7					-0.585*		
18:1-n9			-0.422*				
20:1-n9							0.381*
22:1-n9							0.531*
18:2-n6						-0.542*	
18:3-n3						-0.498*	
EPA		-0.435*					
22:5-n3					0.504*		
DHA						-0.390*	
ARA						-0.402*	
SFA		-0.504*			-0.502*		0.415*
MUFA					-0.516*		
PUFA						-0.510**	
Total n-3						-0.453*	
Total n-6						-0.587**	
n-3/n-6		-0.481*		0.369*			

593 **Table 3**

Fatty acid	VOL1 n= 25	VOL2 n= 15	VOL3 n=29	CON2 n=28	CON3 n=19	MOT2 n=24	MOT3 n=29	P-MOT3 n=18	P-MOT4 n=18	VAP2 n=23	VAP3 n=26
14:0											0.447*
16:0							-0.408*		-0.503*		
16:1											
18:0						-0.451*		0.537*			
18:1-n9							-0.403*		-0.502*		
18:2-n6									-0.604**	0.496*	
18:3-n3			-0.428*		-0.472*				-0.613**	0.540**	
EPA		-0.691**				-0.431*					
22:5-n3	-0.397*			-0.442*	-0.465*						
DHA										0.415*	
ARA											-0.424*
SFA							-0.371*		-0.492*		
MUFA			-0.369*				-0.370*		-0.473*		
PUFA						-0.406*					
Total n-3		-0.515*				-0.413*					
Total n-6									-0.570*	0.426*	
ARA/EPA		0.544*									
EPA/DHA										-0.425*	

594
595 **Table 4**
596

Fatty acid	CON4 n=21	MOT1 n=32	PMOT2 n=16	PMOT3 n=21	VCL4 n=33	VSL3 n=31	VAP3 n=30	VAP4 n=32
14:0					0.432*			
16:0		-0.361*			0.419*			
16:1					0.452**			
18:0		-0.388*		-0.493*		-0.484**		
18:1-n7					0.415*			
18:1-n9					0.427*			
20:1-n9					0.474**			0.381*
22:1-n9			0.499*		0.465**			
18:2-n6					0.390*			
18:3-n3					0.365*			
EPA				-0.454*		-0.418*	0.381*	
22:5-n3		-0.329*		-0.483*				
DHA		-0.370*		-0.438*				
ARA					-0.614**			-0.492**
SFA					0.412*			
MUFA					0.446**			
PUFA		-0.519**		-0.595**		-0.448*		
Total n-3		-0.465**		-0.566**		-0.433*		
ARA/EPA	-0.507**				-0.608*			-0.477**
EPA/DHA							-0.382*	

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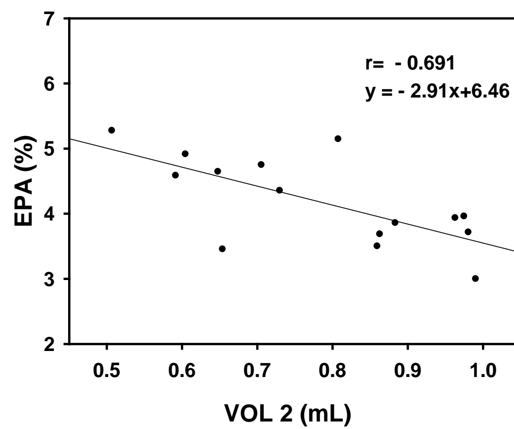
610 **Figure 1**



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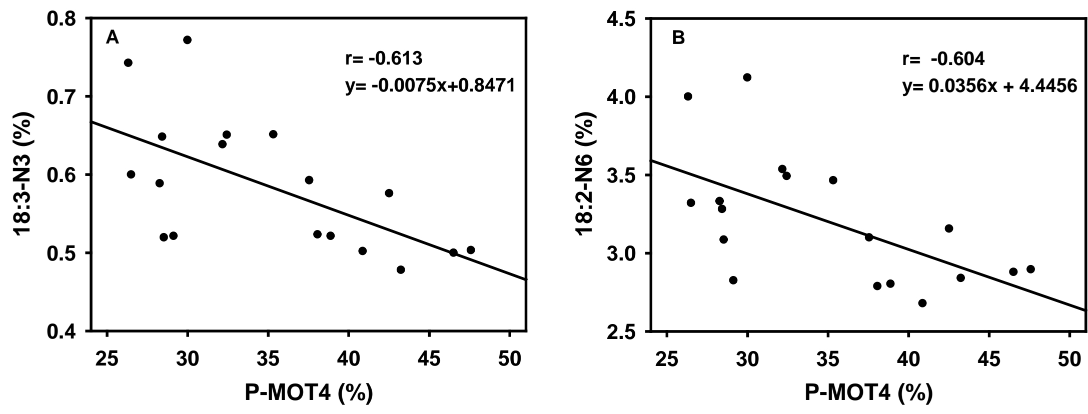
612 **Figure 2**



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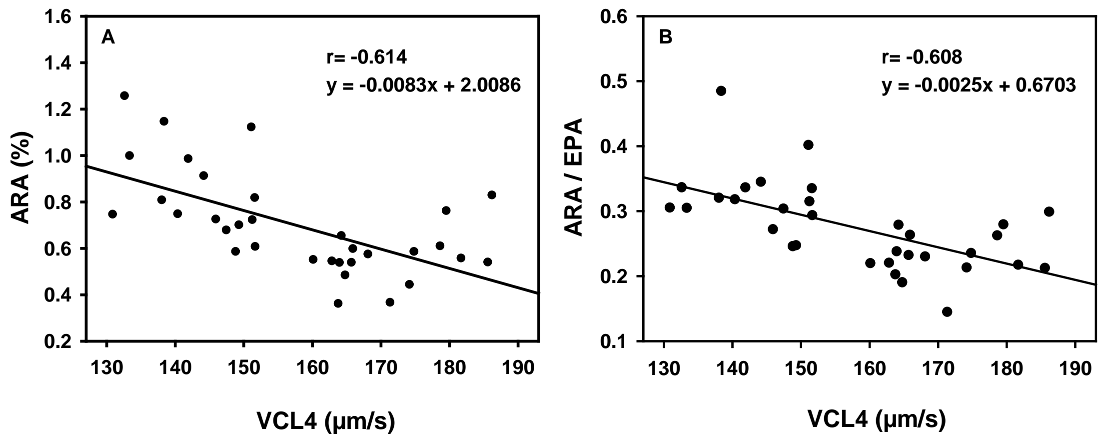
615 **Figure 3**



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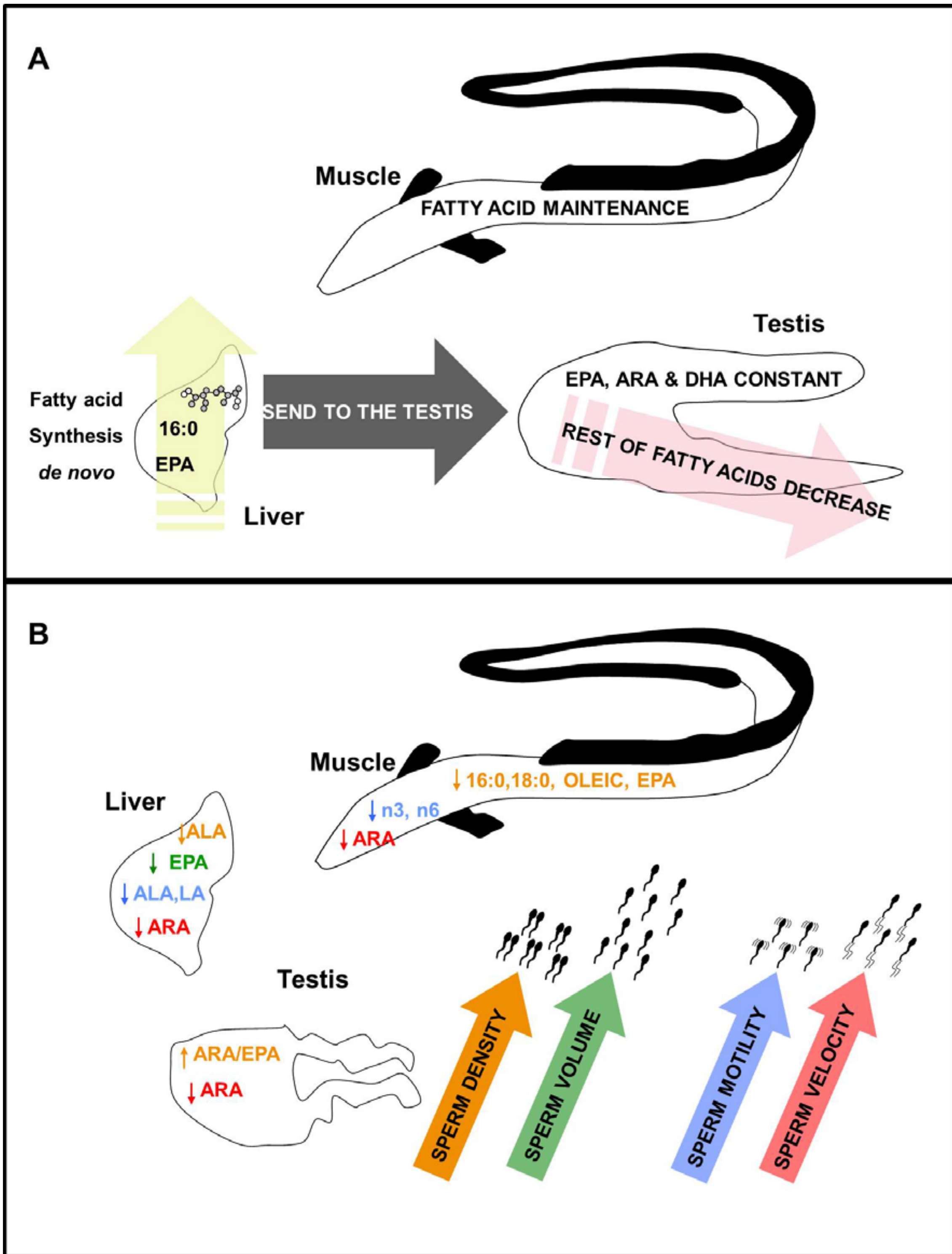
617 **Figure 4**



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