



Effect of different rearing conditions on body lipid composition of greater amberjack broodstock (*Seriola dumerili*)

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1 **Effect of different rearing conditions on body lipid composition of greater amberjack**
2 **broodstock (*Seriola dumerili*).**

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22 **Keywords:** *Seriola dumerili*; broodstock; rearing conditions; stocking density; lipids; fatty
23 acids;

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25 **Abstract**

26 The aim of the present study was to assess the effect of two rearing conditions: outdoor
27 environment with great volume tanks (500 m³) and low stocking density (~0.4 kg/m³);
28 and indoor environment with smaller volume tanks (10m³) and higher stocking density
29 (~5 kg/m³), on muscle, liver and ovary lipid composition of *Seriola dumerili*
30 broodstock born in captivity. The rearing conditions tested seem to affect the pattern of
31 lipid body deposition in broodstock fish of *Seriola dumerili*, increasing the muscle and
32 liver triacylglycerides (TG) accumulation, probably due to a reduced energy
33 expenditure in swimming, with some variations in the fatty acid profile that may
34 respond to the differences in stocking density. No significant differences were found for
35 Gonadosomatic Index (GSI) or ovary lipid deposition between groups in this study,
36 which may suggest that the conditions tested do not have a major effect on ovary
37 development. However, one season later the females kept under outdoor conditions
38 released eggs spontaneously while those kept under indoor conditions did not spawn,
39 suggesting that the conditions tested actually have an effect on the broodstock's
40 reproductive fitness. More studies are need in order to evaluate whether the conditions
41 tested have or not any influence on ovary development.

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50 **Introduction**

51 Greater amberjack (*Seriola dumerili*), a species with increasing interest for aquaculture
52 diversification (Nakada, 2008), has several issues that are hampering its commercial
53 culture, being one of the major concerns to achieve good results of reproduction under
54 captivity conditions. Reproduction is a very complex process that can be affected /
55 modulated by several factors such as nutrition, genetic background or environmental
56 conditions. Regarding nutrition, dietary fatty acids has proven to be very important in
57 the reproduction of several species since determine gonad composition affecting not
58 only sperm and egg quality (Izquierdo et al., 2001; Tocher et al., 2010), but also being
59 involved in the synthesis of eicosanoids, autocrine mediators in the reproductive process
60 (Mercure and Van der Kraak, 1996; Sorbera *et al.*, 2001; Patiño *et al.*, 2003; Tocher *et*
61 *al.*, 2003; Stacey and Sorensen, 2005; Henrotte *et al.*, 2011). Bearing in mind the
62 importance of lipids on breeders' diet, several studies has been performed by our
63 research group in greater amberjack, achieving as a result a broodstock diet which
64 approximates the ovary lipid composition of cultured fish to that from wild specimens
65 (Rodríguez-Barreto *et al.*, 2012; 2014).

66 On the other hand, adequate rearing conditions are also very important to ensure animal
67 welfare. It's well documented the effect of temperature on the reproductive performance
68 of cultured finfish (Portz *et al.*, 2006). Other factors such us fish stocking density and
69 water volume also influence fish physiology and welfare, and may affect reproductive
70 fitness (Ellis *et al.*, 2002; Conte, 2004; Mylonas *et al.*, 2010). Under stressful
71 conditions, trade-offs between reproductive efforts and somatic growth may occur
72 (Schreck *et al.*, 2001). In this regard, each teleost species have a particular response to a
73 given stressor, that also may vary considerably depending on the intensity and duration
74 of the stressor (Schreck, 2010), being the maintenance of body weight at the expense of

75 gonad development, or the maintenance of eggs production at the expense of somatic
76 tissue the two possible strategies adopted by most of the species. Therefore, culture
77 conditions have a direct effect on the pattern of utilization and mobilization of energy
78 reserves (Portz et al., 2006). Thus, depletion in lipid reserves or some specific fatty
79 acids has been observed in several species kept under high stocking densities
80 (Papautsoglou *et al.*, 2006; Karakatsouli *et al.*, 2007; Montero *et al.*, 1999, 2001).

81 Spontaneous spawning under captivity usually needs moderate to large holding volumes
82 and low stocking densities in most fish species, being tank size, water column depth or
83 stocking density parameters that have been proved to influence reproductive success in
84 some cultured fish (Mylonas *et al.*, 2010). In fact, despite the difficulties encountered
85 with the reproduction of greater amberjack (Kozul et al., 2001; Lazzari et al., 2000;
86 Mylonas *et al.*, 2004), it have been shown that wild greater amberjack broodstock
87 maintained in large volume raceways tanks, can reach sexual maturation and spawn
88 spontaneously for several consecutive years (Jerez et al. 2006; 2007).

89 Taking all these considerations into account, and given the absence of trials with
90 broodstock in which different culture conditions have been tested, the aim of this work
91 was to study the effect of two rearing conditions (outdoor environment with great
92 volume tanks and low stocking density and indoor environment with smaller volume
93 tanks and higher stocking density) on muscle, liver and ovary lipid composition of
94 *Seriola dumerili* broodstock born in captivity in order to assess whether these conditions
95 have any influence on lipid mobilization and ovary development.

96 **Material and Methods**

97 Fish and experimental conditions

98 First generation (F1) greater amberjack (*Seriola dumerili*) broods¹, born in captivity
99 in the experimental culture facilities of the Spanish Institute of Oceanography (Tenerife,
100 Canary Islands, Spain), individually identified with PIT tags, were selected and
101 randomly sorted into two groups (2 tanks per group) and kept under different rearing
102 conditions.

103 Fish of the group henceforth named Outdoor, were randomly distributed in two outdoor
104 high capacity raceway tanks (500 m³) with low density (20 fish per tank, with $9.51 \pm$
105 2.86 kg mean weight, ~ 0.4 kg/m³). The tanks were maintained with continuous water
106 supply of 2100 L·min⁻¹ to ensure oxygen level close to saturation, natural seawater
107 temperature ranging between 19.4 °C and 23.7 °C, and natural photoperiod. Natural
108 sunlight intensity was attenuated by tank covers.

109 Fish of the other group hereafter called Indoor, were randomly placed in two indoor
110 lower volume polyethylene square tanks (10 m³), with higher density (6 fish per tank,
111 with 8.07 ± 2.00 kg mean weight, ~ 5 kg/m³). The tanks had a continuous seawater
112 supply of 80 L·min⁻¹, oxygen level close to saturation, natural water temperature ranging
113 between 19.4 °C and 23.7 °C, and natural photoperiod. These tanks were located inside
114 a warehouse.

115 Both groups of fish were fed with a diet previously designed by our group (Rodríguez-
116 Barreto *et al.*, 2014) (SPAROS, Algarve, Portugal), (Diets Composition Table 1) during
117 7 month (February to August). Feed was supplied once a day and three days a week (1%
118 of biomass day⁻¹).

119 In April, when the spawning period begins in similar culture conditions (Jerez *et al.*,
120 2006), each tank was fitted with an overflow egg collector and checked daily.

121 Sampling methods

122 In August, during the second  of the spawning season, three mature females of
123 *Seriola dumerili* per tank (n=6) were randomly selected and sacrificed in order to
124 compare their lipid profile of muscle, liver and ovary. After the sacrifice by an
125 anesthetic overdose (2-phenoxiethanol, 600 ppm), gonadal maturity was confirmed by
126 visual examination (Holden and Raitt, 1974). Biometric parameters of length, and body,
127 gonad and liver weight were measured. Samples of ovary, liver and muscle tissue were
128 dissected off, frozen in liquid nitrogen and stored at -80°C at -80 °C until lipid analysis.
129 A visual assessment of the organs external appearance and the deg^o of fat deposition
130 in the peritoneal cavity was carried out. Gonadosomatic index (GSI) and hepatosomatic
131 index (HSI) were established using the following formula, respectively: $GSI =$
132 $100(\text{Ovary wt} \cdot \text{body wt}^{-1})$; $HSI = 100(\text{Liver wt} \cdot \text{body wt}^{-1})$ .

133 Assay methods

134 Moisture content was determined in 300- 500 mg samples by thermal drying of samples
135 in an oven at 110°C until constant weight, according to the Official Method of Analysis
136 of the Association of Official Analytical Chemists (AOAC, 1990).

137 Total lipid (TL) was extracted from the tissues and diet by homogenization in
138 chloroform/methanol (2 : 1, v/v) according to the method of Folch et al.(1957). The
139 organic solvent was evaporated under a stream of nitrogen and the lipid content was
140 determined gravimetrically (Christie, 1982) and stored in chloroform/methanol (2 : 1),
141 containing 0.01% butylated hydroxytoluene (BHT). Analysis of lipid class (LC)
142 composition was performed by one-dimensional double development high-performance
143 thin layer chromatography (HPTLC) using methyl
144 acetate/isopropanol/chloroform/methanol/0.25% (w/v) KCl (5 : 5 : 5 : 2 : 1.8, by
145 volume) as developing solvent system for the polar lipid classes and isohexane/diethyl
146 ether/acetic acid (22.5 : 2.5 : 0.25, by volume), for the neutral lipid separation. Lipid

147 classes were visualized by charring with 3% (w/v) aqueous cupric acetate containing
148 8% (v/v) phosphoric acid, and quantified by scanning densitometry using a dual-
149 wavelength flying spot scanner Shimadzu CS-9001PC (Shimadzu, Duisburg, Germany)
150 (Olsen & Henderson 1989). Phosphatidylcholine (PC), phosphatidylethanolamine (PE),
151 and triacylglycerides (TG) were purified by thin layer chromatography (TLC) using the
152 polar solvent system described before for PC and PE purification, and the neutral
153 solvent system for TG. The separated classes were sprayed with 0.1% 2', 7'-
154 diclorofluorescein in methanol (98%) (w/v), containing BHT, and visualized under
155 ultraviolet light. Bands were scraped off the plates into tubes for the subsequent analysis
156 of fatty acids.

157 To determine the fatty acid profiles, TL extracts and PC, PE, and TG fractions were
158 subjected to acid-catalyzed transmethylation with 1% sulphuric acid (v/v) in methanol.
159 The resultant fatty acid methyl esters (FAME) were purified by thin layer
160 chromatography (TLC) (Christie, 1982). During acid-catalyzed transmethylation,
161 FAMES are formed simultaneously with dimethyl acetals (DMAs) which originate from
162 the 1-alkenyl chain of plasmalogens. FAME and DMA were separated and quantified
163 using a TRACE-GC Ultra gas chromatograph (Thermo Scientific) equipped with an on-
164 column injector, a flame ionization detector and a fused silica capillary column,
165 Supelcowax TM 10 (Sigma-Aldrich, Madrid, Spain). Individual FAME and DMA were
166 identified by reference to authentic standards, and further confirmation of FAMES and
167 DMAs identity was carried out by GC-MS (DSQ II, Thermo Scientific).

168 Statistical analysis

169 Results are reported as means \pm standard deviation (SD). Non-detected fatty acids were
170 considered as 0 value for statistical analysis. Normal distribution was checked for all
171 data with the one-sample Kolmogorov-Smirnoff test and homogeneity of the variances

172 with the Levene test. Differences between pairs of means were tested using Student's t-
173 test. In all statistical tests used, $p < 0.05$ was considered significantly different. Statistical
174 analysis was carried out using the IBM SPSS statistics package (version 20.0 for
175 Windows).

176 Results

177 No significant differences were found in the final body weight, HSI or GSI between
178 groups (Table1), however a net reduction in body weight (body final weight - body
179 initial weight) was observed at the end of the experiment in both fish groups when
180 individual data are considered, being slightly higher in fish kept outdoor (Outdoor %
181 weight loss = $9\% \pm 4.62$; Indoor % weight loss = $2.09\% \pm 4.24$).

182 Muscle of those fish kept under indoor conditions displayed higher total lipid content
183 than those kept outdoor, even though there is a great heterogeneity among the indoor
184 group (Figure 1A). Significant differences were found in Total Neutral Lipid (TNL)
185 content, mainly due to differences in the amount of triacylglycerides (TG), while no
186 significant differences were found in Total Polar Lipid (TPL) and each individual
187 phospholipid contents. The high dispersion detected in muscle TL content of indoor fish
188 is due to a high variation in TG accumulation. (Figure 1B)

189 In liver tissue, no significant differences were encountered in TL, TPL and TNL content
190 between groups, although TNL content tended to be lower in fish kept under outdoor
191 conditions (Figure 2A). Some individual lipid class showed a trend, with sphingomyelin
192 (SM), phosphatidylserine (PS), phosphatidylethanolamine (PE), and sterol esters (SE)
193 being slightly higher in outdoor fish than in indoor fish, while the amount of fatty
194 acids (FFA) and TG higher in indoor animals. Only the differences in PS and FFA were
195 statistically significant. As observed in muscle, dispersion in TL content data was
196 detected as a result of the fluctuation in TG accumulation in both groups. (Figure 2B)

197 Ovaries from fish kept under outdoor conditions tended to have a slightly lower level of
198 lipid than those fish kept under indoor ones (Figure 3A). This trend was due to a lower
199 content of TPL, finding significant differences in phosphatidylcholine (PC), and
200 phosphatidylglycerol (PG). TNL also tended to be lower in outdoor group than in
201 indoor, with a lower content of TG and EE. Despite the differences found in lipid class
202 content (% p.s.), no differences in the relative proportions (%) of TNL and TPL
203 between groups were detected (data not shown).

204 Fatty acid composition of ovary, muscle and liver total lipid extract is shown in Table 2.
205 Regarding fatty acid composition of muscle and liver, no differences in the total
206 percentage of saturates, monounsaturated fatty acids (MUFA), polyunsaturated fatty
207 acids (PUFA) and n-3 HUFA (Highly unsaturated fatty acids) were found between both
208 groups, however some differences were encountered in the relative proportions of
209 certain fatty acids. Among saturates, fish kept under indoor conditions displayed higher
210 proportions of 14:0 and 15:0 and lower proportions of 18:0 than fish kept under outdoor
211 conditions. With regard to MUFA, some minor differences were found for 16:1, and
212 18:1 n-7 in both tissues with indoor fish showing higher proportions of those fatty acids.
213 Also marginal differences were detected for 17:1 n-7 and 20:1 n-7 in muscle, and for
214 22:1 in liver. In muscle, the most striking difference among PUFA is the higher level of
215 18:2 n-6 in outdoor animals with respect to indoor ones. Muscle n-6 HUFA were
216 significantly higher in outdoor fish, identifying the same trend in liver. 20:4 n-6 (ARA,
217 arachidonic acid) proportions as well as other 22 C n-6 HUFA tended to be higher in
218 outdoor conditions. Although no significant differences in the total level of n-3 HUFA
219 were found between groups, the relative proportion of 20:4 n-3, 20:5 n-3 (EPA,
220 eicosapentanoic acid) and 21:5 n-3 was significantly higher in fish kept under indoor
221 conditions, while opposite trend was observed for 22:6 n-3 (DHA, docosahexanoic acid)

222 which tended to be higher in outdoor fish although they were not significantly different.
223 The noted differences in EPA, and the observed trends in ARA and DHA conducted to
224 remarkable differences in EPA/ARA and DHA/EPA ratios.

225 In relation to ovary fatty acid profile, differences in the total level of saturated, PUFA,
226 n-6 and n-3 HUFA were detected. The cumulative proportion of saturates was higher in
227 outdoor conditions than in indoor ones, primarily due to the higher accumulation of
228 16:0. 18:2 n-6, as other 18 C PUFA, tended to be higher in the ovary of fish kept under
229 indoor conditions. Among HUFA, n-6 proportion was larger in outdoor fish, and n-3
230 proportion was higher in indoor fish. Although, no significant differences were found
231 for the most relevant fatty acids, the following trends were observed: ARA displayed
232 higher proportions under the outdoor setting than under indoor conditions, and EPA
233 seems to be lower in outdoor fish than in indoor ones. These tendencies led to a
234 significantly lower EPA/ARA ratio in outdoor conditions.

235 Fatty acid composition of ovary, muscle, and liver phosphatidylcholine (PC),
236 phosphatidylethanolamine (PE) and triacylglycerides (TG) fractions are shown in
237 Tables 3, 4 and 5 respectively. Concerning to muscle and liver PC, PE and TG fatty acid
238 profile, some generalizations can be done. Thus, the phospholipids analyzed (PC and
239 PE) showed the following trends: A higher level of saturates in the indoor group, and a
240 higher proportion of MUFA (18:1 n-9), and PUFA (18:2 n-6) in the outdoor group. PC
241 tended to display a higher level of EPA in indoor fish while this fatty acid tended to be
242 higher in outdoor group in muscle PE. MUFA and PUFA of TGs from muscle and liver
243 showed the opposed trends described for PC and PE. TG n-3 HUFA level was higher in
244 indoor fish.

245 When the fatty acid profile of ovary lipid class was considered, some minor differences
246 were found between groups in PC, without significant differences for the major and

247 more relevant fatty acids, except for 18:2 n-6, which showed slightly higher proportions
248 in outdoor fish. However, it must be said, that the total level of n-3 HUFA, including
249 EPA and DHA proportions, tended to be higher in indoor fish. In PE, the saturates
250 displayed higher levels in Indoor conditions, while monoenes and some n-6 PUFA as
251 18:2 n-6 were lower in this group than in outdoor one. TG profile of fish kept in indoor
252 conditions was characterized by significantly higher proportions of saturates and MUFA
253 and significantly lower proportions of PUFA, particularly n- 6 and n-3 HUFA than fish
254 kept in outdoor conditions. With those differences in n-3 HUFA, resulting from the
255 noticeable higher proportion of DHA in the outdoor group, what led to an also higher
256 DHA/EPA ratio in this group.

257 **Discussion**

258 Nutrients required for egg production are derived from diet as well from body reserves.
259 During pre-spawning and spawning periods, fish increase their reproductive investment
260 through a depletion of energy reserves that will be channeled into the mass production
261 of roe, and which result in a decrease or cease in body growth. On the other hand, a
262 decrease in food intake is usually observed in mature spawners (Hoskins *et al.*, 2008;
263 Volkoff *et al.*, 2009). Thus, the weight loss observed in this study could respond to a
264 loss of appetite (Volkoff *et al.*, 2009) or/and reallocation of energy from somatic growth
265 to gonadal growth during the spawning period as described for other species such as
266 North Sea plaice (*Pleuronectes platessa*), Atlantic cod (*Gadus Morhua*), gilthead
267 seabream (*Sparus aurata*), Atlantic salmon (*Salmo salar*), European sea bass
268 (*Dicentrarchus labrax*) or Atlantic halibut (*Hippoglossus hippoglossus*) (Kissil *et al.*,
269 2001; Dahle *et al.*, 2003; Rijnsdorp *et al.*, 2005; Karlsen *et al.*, 2006, Taranger *et al.*,
270 2010).

271 Rearing conditions may affect the pattern of energy usage and reserve mobilization.
272 Stocking density is widely recognized as a critical husbandry factor in intensive
273 aquaculture because it represents a potential source of chronic stress that can impact
274 metabolism and growth (Ellis *et al.*, 2002; Portz *et al.*, 2006). Although there are other
275 factors closely related to this, as holding containers design or water quality, that also
276 can act as stressors influencing physiology and behavior of farmed fish (Portz *et al.*,
277 2006). High stocking densities may affect the mobilization of energy reserves, including
278 lipids, in order to cope with the possible demands of energy imposed by this stress
279 situation, altering lipid and fatty acids metabolic pathways. In Papautsoglou *et al.*
280 (2006) and Karakatsouli *et al.* (2007) decreased levels of TL were detected in juveniles
281 of white seabream stocked under high densities, depletion that could be produced in
282 response to the overcoming stressor to satisfy the increased energy demand. In contrast
283 no significant differences in liver total lipid content and TG proportions were found
284 between juveniles of gilthead seabream reared under low and high densities (Montero *et*
285 *al.*, 2001). An increased liver TG accumulation was associated to crowding in juveniles
286 of wedge sole (Herrera *et al.*, 2009) and variations in polar lipid were observed in brill
287 (Herrera *et al.*, 2012) submitted to similar conditions. Therefore the physiological
288 responses to high stocking situations may vary depending on the species. In this study a
289 considerable higher TG accumulation was detected in muscle of females kept under
290 high densities. Furthermore, although no differences were detected in the liver TL
291 content between the two rearing conditions tested, indoor females kept under high
292 stocking density displayed higher free fatty acids content in their liver, with the same
293 trend for TG. As a result of the confinement situation a decrease in the speed of
294 swimming would be expected, with the consequent reduction in locomotor spending
295 (Santos *et al.*, 2010). Thus, the increased triglycerides accumulation at muscular level in

296 fish kept under indoor setting might respond to a decrease in metabolic expenditures for
297 activities such as swimming in this group.

298 The lower proportion of oleic and linoleic acid observed in the fatty acid profile of
299 muscle and liver phospholipids (PC and PE) of fish kept under indoor conditions respect
300 to those kept under outdoor ones is consistent with the role of these fatty acids as energy
301 substrate. In agreement with the results obtained by Montero *et al.* (1999; 2001) with
302 gilthead sea-bream, a reduction in the relative proportion of 18:1 n-9 in liver total lipids
303 was found under high stocking density and discussed in relation to an increased energy
304 demand in crowding stress situation. Some studies have shown a decrease in HUFA
305 values in the fish held on high density, either in the polar lipid (Montero *et al.*, 1999) or
306 in the total lipid extract (Karakoutsouli *et al.*, 2007). Contrary to those results we found
307 increased levels of EPA in muscle and liver total lipid of fish kept under high
308 confinement, probably due to the higher accumulation of this fatty acid in TGs,
309 although it must be said that a clear reduction in n-3 HUFA (EPA and DHA) was
310 observed in muscular PE. It may suggest that some compensatory mechanisms were
311 involved. In addition, there were significant differences in other fatty acids in total
312 lipids, although they did not show a clear pattern of conduct.

313 As discussed above, a reallocation of energy from somatic growth to gonadal growth
314 take place surrounding reproduction. However stress may involve a redistribution of
315 metabolic energy what could negatively interferes with other physiological processes
316 and may affect fish reproductive fitness (Schreck *et al.*, 2001; Barton, 2002; Potrtz *et*
317 *al.*, 2006). In the case of study, no significance differences were found in GSI, or ovary
318 total lipid content between fish kept under both rearing conditions. Further to this, none
319 of the groups spawn during the present study. This may suggest that the tested
320 conditions may not have a major effect on ovary development, at least after 7 month of

321 trial. However, it is worth to mention that one season later the animals kept under
322 outdoor conditions released eggs spontaneously (Rodríguez-Barreto *et al.*, 2014) while
323 those kept under indoor conditions did not. The fact that spontaneous spawn of this
324 species only have been achieved when fish were kept under low density into high
325 capacity tanks (Jerez *et al.*, 2006; 2007), may suggest that the conditions tested actually
326 have an effect on the broodstock's reproductive fitness, as it has been shown for other
327 species which also require large holding volumes and low stocking densities to
328 effectively reproduce under captivity (Mylonas *et al.*, 2010). Further studies should be
329 done in order to confirm or rebut this hypothesis, and to optimize tank size and water
330 depth to obtain successful spawning in *Seriola dumerili* cultured broodstock.

331 It is also worth noting that although fish kept under indoor conditions showed a higher
332 body lipid reserve, it was not translated into a significantly higher lipid mobilization to
333 gonad development. This is concordant with other studies where reduced exercise has
334 had an effect on body lipid deposition, but has not resulted in energy reallocation for
335 reproduction (Patterson *et al.*, 2004; Karlsen *et al.*, 2006). In vertebrates, in some cases,
336 excess energy storage may inhibit reproduction despite a high body fat content and high
337 plasma concentrations of hormones that are thought to stimulate reproductive processes
338 (Shneider *et al.*, 2004).

339 Regarding ovary lipid composition, lipid class and fatty acid profiles showed some
340 differences between groups. The most striking difference among lipid class was the
341 higher content of PC in animals held under indoor conditions, that drive to a higher
342 content of polar lipid, even though, this group also tended to exhibit an increased
343 accumulation of neutral lipid, particularly TG and EE, although there were no
344 differences in relative  proportion of NL and PL (%).

345 It has long been known that body tissue fatty acid composition is comprehensively
346 dependent on diet composition, and can be modified by several factors, nevertheless,
347 some fatty acids seem to be selectively retained within certain limits depending on fish
348 species, diet or the nature of the tissue where they are accumulated. In this regard, eggs,
349 and consequently the ovary tissue where they derive from, are more resistant to
350 variations in their fatty acid profile than other tissues, and the levels of some fatty acids
351 such as DHA tend to be conserved selectively independently to other factors such as
352 rearing conditions or dietary regime (Sargent *et al.*, 2002; Tocher *et al.*, 2010).
353 Although some differences were detected for some fatty acids, including an increment
354 on the total level of saturates (TL, PE and TG), no differences were found for the most
355 relevant fatty acids in the total lipid extract of ovary tissue. However, it is remarkable
356 the higher DHA relative proportion, and consequently DHA/EPA ratio in ovary TG
357 from fish kept outdoor. Given the slightly lower content of TG in this group, the
358 differences observed may be a compensatory response in order to maintain the total
359 amount of this essential fatty acid.

360 One could speculate whether the results obtained under indoor and outdoor conditions
361 are just the result of differences in the stocking densities and swimming activities
362 between the two rearing systems, or if there are other factors that also may influence in
363 some extent the parameters studied. Such factors are difficult to analyze by themselves,
364 since, for instance, tank size is correlated to water volume, and may also influence water
365 flow rate and quality, all parameters that can influence reproductive success (Mylonas *et*
366 *al.*, 2010). The presence /absence of tank covers as well as depth of water column or
367 light conditions may all have had an influence on the results obtained, since under
368 outdoor conditions animals are submitted upon natural photoperiod cycle, with daily
369 and seasonal changes in light intensity and spectral composition while in indoor tanks

370 light is kept at a more constant intensity throughout the year, thus fish experienced a
371 different ambient light in both treatments.

372 In summary, we can conclude that the rearing conditions tested seem to affect the
373 pattern of lipid body deposition in broodstock fish of *Seriola dumerili*, increasing the
374 muscle and liver TG accumulation, probably due to a reduced energy expenditure in
375 swimming, with some variations in the fatty acid profile that may respond to the
376 differences in stocking density. But those differences on body lipid reserves, does not
377 appear to have a clear effect on ovary development, since no significant differences
378 were found for GSI or lipid deposition between groups.

379 Further studies will be necessary in order to determine the optimal conditions for
380 broodstock culture in this species, with special effort on the influence of factors such as
381 handling stress, photoperiod, illumination environment, stocking density or even
382 holding containers design on *Seriola dumerili* reproduction, since reproduction still
383 remains as an important bottleneck in the culture of this species.

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For Review Only

389 **References**

- 390 Barton, B. A. (2002) Stress in fishes: a diversity of responses with particular reference
391 to changes in circulating corticosteroids. *Integrative and Comparative Biology*,
392 42(3), 517-525.
- 393 Conte, F. S. (2004) Stress and the welfare of cultured fish. *Applied Animal Behaviour*
394 *Science* 86(3), 205-223.
- 395 Dahle, R., Taranger, G. L., Karlsen, Ø., Kjesbu, O. S., & Norberg, B. (2003) Gonadal
396 development and associated changes in liver size and sexual steroids during the
397 reproductive cycle of captive male and female Atlantic cod (*Gadus morhua*
398 L.). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative*
399 *Physiology* 136(3), 641-653.
- 400 Ellis, T., North, B., Scott, A. P., Bromage, N. R., Porter, M., & Gadd, D. (2002) The
401 relationships between stocking density and welfare in farmed rainbow
402 trout. *Journal of Fish Biology* 61(3), 493-531.
- 403 Henrotte, E., Milla, S., Mandiki, S. N. M., & Kestemont, P. (2011) Arachidonic acid
404 induces production of 17,20b-Dihydroxy-4-pregnen-3-one (DHP) via a putative
405 PGE2 receptor in fish follicles from the eurasian perch. *Lipids* 46, 179–187.
- 406 Herrera, M., Vargas-Chacoff, L., Hachero, I., Ruíz-Jarabo, I., Rodiles, A., Navas, J. I.,
407 & Mancera, J. M. (2009) Physiological responses of juvenile wedge sole
408 *Dicologlossa cuneata* (Moreau) to high stocking density. *Aquaculture*
409 *Research* 40(7), 790-797.
- 410 Herrera, M., Ruiz-Jarabo, I., Hachero, I., Vargas-Chacoff, L., Amo, A., & Mancera, J.
411 M. (2012) Stocking density affects growth and metabolic parameters in the brill
412 (*Scophthalmus rhombus*). *Aquaculture International* 20(6), 1041-1052.
- 413 Hoskins, L. J., Xu, M., & Volkoff, H. (2008) Interactions between gonadotropin-
414 releasing hormone (GnRH) and orexin in the regulation of feeding and
415 reproduction in goldfish (*Carassius auratus*). *Hormones and behavior* 54(3),
416 379-385.
- 417 Izquierdo, M.S., Fernández-Palacios, H., & Tacon, A.G.J. (2001) Effect of broodstock
418 nutrition on reproductive performance of fish. *Aquaculture* 197, 25–42.

- 419 Jerez, S., Samper, M., Santamaría, F.J., Villamandos, J.E., Cejas, J.R., & Felipe, B.C.
420 (2006) Natural spawning of greater amberjack (*Seriola dumerili*) kept in
421 captivity in the Canary Islands. *Aquaculture* 252, 199-207.
- 422 Jerez, S., Cejas, J.R., Villamandos, J.E., Samper, M., Felipe, B.C. & Santamaría, F.J.
423 (2007) Comportamiento reproductivo y calidad de puesta de reproductores de
424 *Seriola dumerili* entre 2002 y 2006. Actas XI Congreso Nacional de Acuicultura,
425 Vigo, España, pp.787-790.
- 426 Karakatsouli, N., Papoutsoglou, S. E., & Manolessos, G. (2007) Combined effects of
427 rearing density and tank colour on the growth and welfare of juvenile white sea
428 bream *Diplodus sargus* L. in a recirculating water system. *Aquaculture*
429 *Research* 38(11), 1152-1160.
- 430 Karlsen, Ø., Norberg, B., Kjesbu, O. S., & Taranger, G. L. (2006) Effects of
431 photoperiod and exercise on growth, liver size, and age at puberty in farmed
432 Atlantic cod (*Gadus morhua* L.). *ICES Journal of Marine Science* 63(2), 355-
433 364.
- 434 Kissil, G. W., Lupatsch, I., Elizur, A., & Zohar, Y. (2001) Long photoperiod delayed
435 spawning and increased somatic growth in gilthead seabream (*Sparus*
436 *aurata*). *Aquaculture* 200(3), 363-379.
- 437 Kozul, V., Skaramuca, B., Glamuzina, B., Glavic, N., & Tutman, P. (2001)
438 Comparative gonadogenesis and hormonal induction of spawning of cultured
439 and wild mediterranean amberjack (*Seriola dumerili*, Risso 1810). *Scientia*
440 *Marina* 65 (3), 215–220.
- 441 Lazzari, A., Fusari, A., Boglione, A., Marino, G., & Di Francesco, M. (2000) Recent
442 advances in reproduction and rearing aspects of *Seriola dumerilii*. In: B. Basurco
443 (Ed.), Recent advances in Mediterranean aquaculture finfish species
444 diversification. Cahiers Options Méditerranéennes, CIHEAM, Zaragoza, Spain,
445 vol. 47, pp. 241–247.
- 446 Mercure, F., & Van Der Kraak, G. (1996) Mechanisms of action of free arachidonic
447 acid on ovarian steroid production in the goldfish. *General Comparative*
448 *Endocrinology* 102, 130–140.

- 449 Montero, D., Izquierdo, M. S., Tort, L., Robaina, L., & Vergara, J. M. (1999) High
450 stocking density produces crowding stress altering some physiological and
451 biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. *Fish*
452 *Physiology and Biochemistry* 20(1), 53-60.
- 453 Montero, D., Robaina, L. E., Socorro, J., Vergara, J. M., Tort, L., & Izquierdo, M. S.
454 (2001) Alteration of liver and muscle fatty acid composition in gilthead
455 seabream (*Sparus aurata*) juveniles held at high stocking density and fed an
456 essential fatty acid deficient diet. *Fish Physiology and Biochemistry* 24(1), 63-
457 72.
- 458 Mylonas, C.C., Papandroulakis, N., Smboukis, A., Papadaki, M., & Divanach, P. (2004)
459 Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using
460 GnRH α implants. *Aquaculture* 237, 141–154.
- 461 Mylonas, C.C., Fostier, A., & Zanuy, S. (2010) Broodstock management and hormonal
462 manipulations of fish reproduction. *General and Comparative*
463 *Endocrinology* 165(3), 516-534.
- 464 Nakada, M. (2008) Capture-based aquaculture of yellowtail. In A. Lovatelli; P.F.
465 Holthus (eds). Capture-based aquaculture. Global overview. FAO Fisheries
466 Technical. Paper. No. 508. Rome, FAO. pp. 199–215.
- 467 Olsen, R.E., Henderson, R.J. (1989) The rapid analysis of neutral and polar marine
468 lipids using double-development HPTLC and scanning densitometry. *Journal of*
469 *Experimental Marine Biology and Ecology* 129, 189-197.
- 470 Papoutsoglou, S. E., Karakatsouli, N., Pizzonia, G., Dalla, C., Polissidis, A., &
471 Papadopoulou-Daifoti, Z. (2006) Effects of rearing density on growth, brain
472 neurotransmitters and liver fatty acid composition of juvenile white sea bream
473 *Diplodus sargus* L. *Aquaculture Research* 37(1), 87-95.
- 474 Patiño, R., Yoshizaki, G., Bolamba, D., & Thomas, P. (2003) Role of arachidonic acid
475 and protein kinase C during maturation-inducing hormone-dependent meiotic
476 resumption and ovulation in ovarian follicles of Atlantic croaker. *Biology of*
477 *Reproduction* 68, 516–523.
- 478 Patterson, D. A., Macdonald, J. S., Hinch, S. G., Healey, M. C., & Farrell, A. P. (2004)
479 The effect of exercise and captivity on energy partitioning, reproductive

- 480 maturation and fertilization success in adult sockeye salmon. *Journal of Fish*
481 *Biology* 64(4), 1039-1059.
- 482 Portz, D. E., Woodley, C. M., & Cech Jr, J. J. (2006) Stress-associated impacts of short-
483 term holding on fishes. *Reviews in Fish Biology and Fisheries* 16(2), 125-170.
- 484 Rijnsdorp, A. D., Grift, R. E., & Kraak, S. B. (2005). Fisheries-induced adaptive change
485 in reproductive investment in North Sea plaice (*Pleuronectes platessa*)?
486 *Canadian Journal of Fisheries and Aquatic Sciences* 62(4), 833-843.
- 487 Rodríguez-Barreto, D., Jerez, S., Cejas, J. R., Martin, M. V., Acosta, N. G., Bolaños, A.,
488 & Lorenzo, A. (2012) Comparative study of lipid and fatty acid composition in
489 different tissues of wild and cultured female broodstock of greater amberjack
490 (*Seriola dumerili*). *Aquaculture* 360, 1-9.
- 491 Rodríguez-Barreto, D., Jerez, S., Cejas, J. R., Martin, M., Acosta, N. G., Bolaños, A., &
492 Lorenzo, A. (2014) Ovary and egg fatty acid composition of greater amberjack
493 broodstock (*Seriola dumerili*) fed different dietary fatty acids profiles. *European*
494 *Journal of Lipid Science and Technology* 116(5), 584-595.
- 495 Santos, G. A., Schrama, J. W., Mamauag, R. E. P., Rombout, J. H. W. M., & Verreth, J.
496 A. J. (2010) Chronic stress impairs performance, energy metabolism and welfare
497 indicators in European seabass (*Dicentrarchus labrax*): the combined effects of
498 fish crowding and water quality deterioration. *Aquaculture* 299(1), 73-80.
- 499 Sargent, J.R., Tocher, D.R., & Bell, J.G. (2002) The lipids. In: Halver, J.E., Hardy,
500 R.W. (Eds.), *Fish Nutrition*, 3rd ed. Elsevier, USA, pp. 181– 257.
- 501 Schreck, C.B., Contreras-Sanchez, W., & Fitzpatrick, M.S. (2001) Effects of stress on
502 fish reproduction, gamete quality, and progeny. *Aquaculture* 197, 3–24.
- 503 Schreck, C.B. (2010) Stress and fish reproduction: The roles of allostasis and hormesis.
504 *General and Comparative Endocrinology* 165, 549–556.
- 505 Schneider, J. E. (2004) Energy balance and reproduction. *Physiology & Behavior*
506 81(2), 289-317.
- 507 Sorbera, L.A., Asturiano, J.F., Carrillo, M., & Zanuy, S. (2001) Effects of
508 polyunsaturated fatty acids and prostaglandins on oocyte maturation in a marine
509 teleost, the European sea bass (*Dicentrarchus labrax*). *Biology of Reproduction*
510 64, 382–389.

- 511 Stacey, N.E. & Sorensen, P.W. (2005) Reproductive pheromones, In: Sloman KA,
512 Wilson RW, Balshine S. (Eds.), Behavior and Physiology of Fish. Academic
513 Press, London, pp. 359-412.
- 514 Taranger, G. L., Carrillo, M., Schulz, R. W., Fontaine, P., Zanuy, S., Felip, A., &
515 Hansen, T. (2010) Control of puberty in farmed fish. *General and Comparative*
516 *Endocrinology* 165(3), 483-515.
- 517 Tocher, D.R. (2003) Metabolism and functions of lipids and fatty acids in teleost fish.
518 *Reviews in Fisheries Science* 11, 107–184.
- 519 Tocher, D.R. (2010) Fatty acid requirements in ontogeny of marine and freshwater fish.
520 *Aquaculture Research* 41, 717-732.
- 521 Volkoff, H., Xu, M., MacDonald, E., & Hoskins, L. (2009) Aspects of the hormonal
522 regulation of appetite in fish with emphasis on goldfish, Atlantic cod and winter
523 flounder: notes on actions and responses to nutritional, environmental and
524 reproductive changes. *Comparative Biochemistry and Physiology Part A:*
525 *Molecular & Integrative Physiology* 153(1), 8-12.

Figure 1.(A)Total lipid (LT), total neutral lipid (TNL), total polar lipid (TPL) and (B) lipid class composition (% dry weight of tissue) of muscle of cultured *Seriola dumerili* reared in outdoor (pale grey bars) and indoor(dark grey bars) conditions. Bars marked with an asterisk (*) show significant differences ($p<0.05$) between pairs of means compared by Student's t-test.

Figure 2.(A)Total lipid (LT), total neutral lipid (TNL), total polar lipid (TPL) and (B) lipid class composition (% dry weight of tissue) of liver of cultured *Seriola dumerili* reared in outdoor (pale grey box) and indoor(dark grey box) conditions. Bars marked with an asterisk (*) show significant differences ($p<0.05$) between pairs of means compared by Student's t-test.

Figure 3.(A)Total lipid (LT), total neutral lipid (TNL), total polar lipid (TPL) and (B) lipid class composition (% dry weight of tissue) of ovaries of cultured *Seriola dumerili* reared in outdoor (pale grey box) and indoor(dark grey box) conditions. Bars marked with an asterisk (*) show significant differences ($p<0.05$) between pairs of means compared by Student's t-test.

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Table 1. Biometric parameters and total lipid content (% dry weight) of sacrificed *Seriola dumerili* specimens reared in indoor and outdoor conditions.

	<i>Indoor</i>	<i>Outdoor</i>
Body initial wt (kg)	8.03±2.73	8.83± 3.39
Body final wt (kg)	7.93±2.93	8.05±3.25
HSI	0.93±0.11	0.87±0.19
GSI	1.86±0.49	1.04±0,36
Liver TL (% dry wt)	33.18±5.01	28.10±8.83
Muscle TL (% dry wt)	23.49±12.73	5.43±1,73 *
Ovary TL (% dry wt)	10.95±0.53	6.32±3.42

Results are expressed as means ± SD (n=6). Values marked with an asterisk (*) show significant differences ($p < 0.05$) between pairs of means, compared by Student's t-test. wt, weight; HSI, hepatosomatic index; GSI gonadosomatic index; TL, total lipid.

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Table 2. Fatty acid composition (% total fatty acids) of ovary, muscle and liver total lipid extract from cultured *Seriola dumerili* specimens reared in indoor and outdoor conditions.

Fatty acids	Muscle		Liver		Ovary	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Saturates^a	25.93±0.43	26.53±3.34	22.34±0.26	23.47±1.78	20.89±0.73	24.77±1.48 *
14:0	4.46±0.32	2.72±0.50 *	2.37±0.32	1.56±0.24 *	0.91±0.11	0.81±0.18
15:0	0.41±0.01	0.27±0.02 *	0.39±0.02	0.29±0.04 *	0.23±0.01	0.23±0.03
16:0	15.89±0.48	15.83±1.20	15.62±0.34	15.23±0.78	15.45±0.28	17.34±0.53 *
17:0	0.54±0.01	0.64±0.14	0.63±0.01	0.74±0.21	0.44±0.09	0.71±0.11 *
18:0	4.42±0.25	6.46±1.60	2.70±0.27	4.69±0.83 *	3.82±0.56	5.31±1.11
20:0	tr	0.31±0.03 *	nd	tr±0.03 *	tr	tr *
22:0	nd	tr *	nd	tr	nd	tr *
24:0	nd	tr *	nd	nd	nd	tr
MUFA^a	34.85±0.74	34.08±4.21	41.06±1.74	40.18±5.54	28.53±1.56	26.81±2.99
16:1^b	7.12±0.62	3.87±1.05 *	4.54±0.65	3.03±0.42 *	2.27±0.19	1.80±0.50
17:1 n-7	0.34±0.01	0.26±0.04 *	0.31±0.02	0.28±0.05	tr	tr
18:1 n-9	20.85±0.82	24.51±4.59	29.61±1.46	30.95±5.40	20.96±1.32	19.44±2.52
18:1 n-7	3.04±0.04	2.71±0.13 *	4.36±0.26	3.54±0.14 *	3.85±0.10	4.00±0.22
20:1^b	2.46±0.20	1.74±0.03 *	1.56±0.22	1.60±0.21	0.94±0.04	0.84±0.10
22:1^b	0.96±0.04	0.92±0.04	0.40±0.04	0.65±0.10 *	0.32±0.05	0.41±0.07
24:1 n-9	nd	tr	nd	nd	nd	tr *
PUFA^a	38.02±0.81	37.97±2.02	36.54±1.85	36.50±3.99	48.15±0.86	44.03±0.74 *
16:2 n-4	0.66±0.09	0.24±0.08 *	0.22±0.04	tr *	nd	nd
16:2 n-3	tr	tr	0.28±0.02	0.26±0.04	0.34±0.05	0.35±0.03
16:3 n-4	0.76±0.11	0.29±0.15 *	tr	nd	nd	nd
18:2 n-6	7.51±0.31	9.99±1.35 *	13.31±1.00	12.40±1.42	9.26±1.17	7.93±1.88
18:2 n-4	0.24±0.02	nd *	nd	tr *	tr	tr
18:3 n-6	tr	nd *	tr	nd *	tr	nd
18:3 n-4	tr	tr	tr	tr *	nd	nd
18:3 n-3	1.44±0.08	1.95±1.37	3.22±0.30	3.25±0.38	1.59±0.27	1.13±0.46
18:4 n-3	0.97±0.13	0.38±0.06 *	0.44±0.03	0.31±0.03 *	tr	tr
n-6 HUFA^a	1.07±0.08	2.09±0.44 *	1.64±0.16	2.19±0.55	4.07±0.25	6.22±1.78 *
20:2 n-6	tr	tr *	0.33±0.02	0.30±0.03	0.21±0.03	0.28±0.04
20:3 n-6	nd	tr	nd	tr	nd	tr *
20:4 n-6	0.64±0.05	1.02±0.27	0.95±0.16	1.30±0.43	2.97±0.19	4.52±1.39
22:4 n-6	nd	tr *	nd	tr *	tr	0.26±0.07 *
22:5 n-6	0.29±0.03	0.57±0.09 *	0.36±0.02	0.40±0.05	0.75±0.04	1.05±0.28
n-3 HUFA^a	24.83±0.72	22.78±2.86	17.34±0.94	18.13±5.05	32.52±1.98	28.23±0.98 *
20:3 n-3	nd	nd	tr	tr	nd	tr
20:4 n-3	0.76±0.04	0.38±0.08 *	0.67±0.04	0.35±0.05 *	0.25±0.01	0.18±0.03
20:5 n-3	7.91±0.90	3.94±0.53 *	5.50±0.97	3.86±0.97	4.88±0.63	3.91±0.11
21:5 n-3	0.48±0.05	0.26±0.04 *	0.28±0.02	tr *	tr	tr
22:5 n-3	3.54±0.12	3.03±1.08	2.28±0.27	1.52±0.49	2.40±0.13	1.94±0.22 *
22:6 n-3	12.14±0.96	15.18±2.24	8.52±0.58	12.15±3.58	24.87±1.49	22.14±1.26
DMAs	tr	tr	nd	nd	2.25±0.14	1.70±0.57
16:0_{DMA}	tr	nd	nd	nd	1.35±0.08	nd *
18:0_{DMA}	0.04±0.07	0.13±0.12	nd	nd	0.40±0.03	0.84±0.26
18:1 n-9_{DMA}	nd	nd	nd	nd	0.41±0.05	0.86±0.32
18:1 n-7_{DMA}	nd	nd	nd	nd	0.10±0.01	nd *
Ratios						
DHA/EPA^d	1.56±0.30	3.89±0.66 *	1.58±0.24	3.13±0.32 *	5.14±0.54	5.67±0.39
EPA/ARA^d	12.42±1.82	3.97±0.58 *	5.80±0.58	3.05±0.34 *	1.64±0.12	0.93±0.34 *

Results are expressed as means ± SD (n=6). Values marked with an asterisk (*) show significant differences (p < 0.05) between pairs of means corresponding to outdoor and indoor rearing conditions in each tissue, compared by Student's t-test. tr, values ≤ 0.20%. nd, not detected. MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; HUFA: Highly polyunsaturated fatty acid; DMAs: Dimethylacteals.

^a Include some minor components not shown in the table.

^b Includes *n*-9 and *n*-7 isomers.

^c Includes *n*-11, *n*-9 and *n*-7 isomers.

^d DHA/EPA, 22: 6 *n*-3/ 20: 5 *n*-3; EPA/ARA, 20: 5 *n*-3/ 20:4 *n*-6.

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Table 3. Fatty acid composition (% total fatty acids) of ovary, muscle and liver phosphatidylcholine fraction from cultured *Seriola dumerili* reared in indoor and outdoor conditions.

Fatty acids	Muscle		Liver		Ovary	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
saturates ^c	37.78±0.77	34.23±1.50 *	28.54±1.25	28.13±0.11 *	32.99±3.44	33.75±2.06
14:0	0.49±0.06	0.32±0.08 *	0.82±0.26	0.73±0.25	0.83±0.16	0.93±0.24
16:0	32.35±0.79	30.18±1.31	21.95±0.95	21.19±1.06 *	28.01±3.65	29.19±2.05
18:0	3.90±1.27	3.14±0.10	5.31±0.52	5.12±1.14	3.25±0.74	2.31±0.30
MUFA ^c	9.16±0.40	10.94±0.33 *	16.16±0.96	18.54±1.00 *	19.27±1.44	25.14±4.05
16:1 ^b	0.97±0.11	1.23±0.28	1.96±0.31	2.13±0.40	1.92±0.22	2.01±0.25 *
18:1 n-9	6.49±0.37	7.85±0.48 *	10.66±0.69	12.62±0.52 *	13.85±1.08	18.46±3.07
18:1 n-7	1.43±0.16	1.37±0.08	2.91±0.19	2.58±0.20	2.95±0.22	3.54±0.65
PUFA ^c	49.71±1.12	53.79±1.24 *	52.12±1.09	53.02±1.01 *	43.94±4.03	39.30±6.29
18:2 n-6	7.43±0.81	10.97±0.30 *	6.62±0.86	7.89±1.36 *	6.37±0.34	7.30±0.30 *
18:3 n-3	0.76±0.17	1.24±0.14 *	1.56±0.37	1.82±0.38 *	0.87±0.12	0.85±0.19
n-6 HUFA ^c	3.24±0.93	3.48±0.16	2.03±0.21	2.22±0.10	3.21±0.12	4.16±1.14
20:4 n-6	1.96±1.02	1.63±0.02	1.47±0.04	1.44±0.07 *	2.47±0.14	3.42±1.05
22:5 n-6	1.10±0.11	1.42±0.18	0.30±0.26	0.59±0.05	0.51±0.04	0.62±0.10
n-3 HUFA ^c	38.46±1.51	37.11±1.30	42.15±1.82	39.92±2.85 *	34.05±3.76	25.84±6.23
20:5 n-3	6.97±0.63	5.38±0.70 *	8.74±0.30	7.11±0.41	6.72±0.74	4.75±1.14
22:5 n-3	3.14±0.25	2.93±0.63	2.07±0.21	1.78±0.38 *	2.07±0.27	1.71±0.40
22:6 n-3	27.86±0.86	28.30±2.38	30.97±1.44	30.57±3.08	25.06±2.76	19.12±4.75
DMAs	1.01±0.29	0.83±0.21	tr	tr	0.82±0.12	1.35±0.43
DHA/EPA	4.02±0.33	5.35±1.13	3.54±0.11	4.32±0.61	3.73±0.10	4.03±0.35
EPA/ARA	4.15±1.78	3.30±0.47	5.93±0.13	4.93±0.05	2.72±0.26	1.51±0.70

Results are expressed as means ± SD (n=6). Values marked with an asterisk (*) show significant differences (p < 0.05) between pairs of means corresponding to outdoor and indoor rearing conditions in each tissue, compared by Student's t-test. Only most relevant and abundant fatty acids are presented in this table. tr, values ≤ 0.20%. nd, not detected. MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; HUFA: Highly polyunsaturated fatty acid; DMAs: Dimethylacteals.

^a Include some minor components not shown in the table.

^b Includes n-9 and n-7 isomers.

^c Includes n-11, n-9 and n-7 isomers.

^d DHA/EPA, 22: 6 n-3/ 20: 5 n-3; EPA/ARA, 20: 5 n-3/ 20:4 n-6.

Table 4. Fatty acid composition (% total fatty acids) of ovary, muscle and liver phosphatidylethanolamine fraction from cultured *Seriola dumerili* reared in indoor and outdoor conditions.

	Muscle		Liver		Ovary	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
saturates^c	30.85±3.05	22.36±1.23 *	37.80±1.61	30.43±6.07	18.28±0.29	13.73±0.50 *
14:0	0.69±0.47	0.26±0.05	0.45±0.12	tr	tr	0.50±0.07 *
16:0	10.04±1.74	7.22±0.72 *	16.39±0.33	14.17±3.04	9.02±0.34	6.70±0.22 *
18:0	14.83±0.79	13.39±0.65	19.03±1.59	14.38±3.34	6.64±0.55	4.59±0.16 *
MUFA^c	5.01±1.28	9.60±0.72 *	11.97±1.66	16.82±2.91	9.88±0.79	13.45±1.32 *
16:1^b	0.68±0.59	1.29±0.12	0.70±0.20	0.75±0.30	0.27±0.47	1.14±0.24
18:1 n-9	3.20±0.56	4.80±0.24 *	6.60±0.51	8.82±1.03 *	5.62±0.43	7.95±0.88 *
18:1 n-7	1.06±0.12	2.42±0.69 *	3.55±0.52	4.56±0.77	2.74±0.15	3.11±0.14 *
20:1^a	tr	0.50±0.06	0.91±0.03	1.38±0.34	0.46±0.04	0.62±0.10 *
PUFA^c	40.52±3.24	53.93±1.33 *	45.23±0.14	50.27±9.25	48.83±2.43	51.65±0.96
18:2 n-6	2.05±0.28	7.24±1.43 *	3.45±0.70	4.71±0.79	3.13±0.67	5.35±0.41 *
18:3 n-6	2.80±0.75	nd *	0.48±0.15	tr *	0.43±0.15	nd *
18:3 n-4	1.08±0.59	nd *	0.41±0.01	nd *	nd	nd
18:3 n-3	nd	0.77±0.28 *	0.60±0.17	1.07±0.16	tr	0.46±0.04
n-6 HUFA^c	1.40±0.65	3.93±0.46 *	1.76±0.06	2.27±0.79	7.45±0.45	8.06±0.47
20:4 n-6	1.21±0.33	2.37±0.27 *	1.21±0.03	1.32±0.39	6.43±0.50	6.75±0.38
22:5 n-6	tr	1.25±0.09 *	0.56±0.03	0.84±0.37	1.02±0.12	1.26±0.14
n-3 HUFA^c	29.63±5.69	40.54±0.28 *	38.91±0.43	40.11±7.47	36.72±1.98	36.32±1.54
20:5 n-3	3.09±0.70	4.28±0.44 *	4.80±0.13	4.16±0.90	5.74±0.73	5.45±0.26
22:5 n-3	0.70±0.63	1.78±0.12	2.05±0.12	1.73±0.10 *	2.33±0.21	2.01±0.16
22:6 n-3	22.69±5.72	34.30±0.58 *	31.42±0.41	33.38±6.36	28.58±2.82	28.86±1.56
DMAs	6.24±1.16	4.28±1.12	5.44±0.39	4.87±1.02	17.86±1.01	19.68±0.19 *
16:0_{DMA}	5.62±1.03	4.46±1.01	0.65±0.01	0.95±0.32	9.43±0.66	10.24±0.42
18:0_{DMA}	4.65±1.15	4.61±1.08	nd	0.35±0.15 *	3.72±0.27	4.26±0.41
18:1 n-9_{DMA}	3.36±0.26	2.04±0.45 *	0.69±0.01	0.98±0.31	3.65±0.43	3.98±0.55
18:1 n-7_{DMA}	1.53±0.19	1.17±0.21	nd	0.21±0.18	1.06±0.04	1.24±0.09 *
DHA/EPA	7.32±0.26	8.11±0.93	6.56±0.26	8.06±0.40 *	5.07±1.06	5.26±0.43
EPA/ARA	2.58±0.34	1.81±0.09 *	3.97±0.01	3.22±0.28 *	0.89±0.06	0.83±0.05

Results are expressed as means ± SD (n=6). Values marked with an asterisk (*) show significant differences (p < 0.05) between pairs of means corresponding to outdoor and indoor rearing conditions in each tissue, compared by Student's t-test. Only most relevant and abundant fatty acids are presented in this table. tr, values ≤ 0.20%. nd, not detected. MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; HUFA: Highly polyunsaturated fatty acid; DMAs: Dimethylacteals.

^a Include some minor components not shown in the table.

^b Includes n-9 and n-7 isomers.

^c Includes n-11, n-9 and n-7 isomers.

^d DHA/EPA, 22: 6 n-3/ 20: 5 n-3; EPA/ARA, 20: 5 n-3/ 20:4 n-6.

Table 5. Fatty acid composition (% total fatty acids) of ovary, muscle and liver total triacylglycerides fraction from cultured *Seriola dumerili* reared in indoor and outdoor conditions.

Fatty acids	Muscle		Liver		Ovary	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
saturates^c	27.08±1.62	25.38±3.97	21.65±0.19	20.78±0.61	21.37±0.49	18.48±0.84 *
14:0	5.11±0.40	3.87±0.97	2.66±0.27	1.97±0.49	1.54±0.17	0.72±0.21 *
16:0	16.46±1.12	13.46±0.80 *	16.04±0.13	14.60±0.06 *	16.75±0.31	13.63±0.74 *
18:0	4.42±0.26	5.81±1.80	1.99±0.41	3.18±0.86	2.26±0.28	1.92±0.35
MUFA^c	37.36±2.01	44.02±1.35 *	45.49±1.34	48.81±2.97	39.09±1.76	31.51±2.42 *
16:1^b	7.42±0.40	5.13±1.53	5.27±0.40	3.52±0.55 *	3.08±0.18	1.75±0.16 *
18:1 n-9	23.08±1.63	31.26±3.39 *	33.85±0.87	38.08±3.07	27.90±1.47	22.08±1.86 *
18:1 n-7	3.02±0.10	3.05±0.13	3.81±0.21	4.08±0.11	5.95±0.05	5.18±0.42
20:1^a	2.65±0.36	2.28±0.22	1.84±0.20	1.90±0.25	1.38±0.15	1.36±0.22
22:1^b	0.86±0.09	1.22±0.14 *	0.35±0.11	0.78±0.16 *	0.51±0.12	0.66±0.07
PUFA^c	33.47±3.54	30.84±2.65	32.27±1.45	30.02±2.52	37.23±1.53	50.01±3.09 *
18:2 n-6	7.51±0.55	10.63±1.64 *	14.06±1.40	14.95±1.49	9.59±0.89	8.76±0.67
18:3 n-3	1.47±0.20	2.30±1.69	nd	3.71±0.11 *	1.66±0.24	1.51±0.52
18:4 n-3	0.95±0.08	0.49±0.03 *	0.42±0.02	0.37±0.04	0.23±0.04	tr
n-6 HUFA^c	0.73±0.19	1.09±0.29	1.08±0.06	0.84±0.23	1.96±0.14	3.18±0.57 *
20:4 n-6	0.52±0.09	0.59±0.17	0.52±0.06	0.47±0.12	1.10±0.16	1.11±0.10
22:5 n-6	tr	0.32±0.02	0.27±0.03	0.29±0.04	0.68±0.02	2.03±0.48 *
n-3 HUFA^c	21.58±2.76	15.11±0.32 *	12.76±0.62	9.30±2.92	25.13±2.16	35.58±3.84 *
20:5 n-3	7.41±1.04	3.37±0.30 *	4.61±0.46	2.67±0.71 *	2.60±0.39	2.30±0.26
22:5 n-3	3.24±0.47	3.01±1.21	2.23±0.28	1.40±0.53	2.09±0.15	2.60±0.45
22:6 n-3	9.81±1.41	8.05±1.52	4.95±0.60	4.58±1.64	20.06±1.80	30.25±3.41 *
DMAs	nd	nd	nd	nd	nd	nd
DHA/EPA	1.33±0.18	2.43±0.69 *	1.09±0.22	1.72±0.36	7.79±0.98	13.31±2.50 *
EPA/ARA	14.39±1.11	6.05±1.55 *	8.80±0.27	5.75±0.23 *	2.38±0.30	2.09±0.38

Results are expressed as means ± SD (n=6). Values marked with an asterisk (*) show significant differences (p < 0.05) between pairs of means corresponding to outdoor and indoor rearing conditions in each tissue, compared by Student's t-test. Only most relevant and abundant fatty acids are presented in this table. tr, values ≤ 0.20%. nd, not detected. MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; HUFA: Highly polyunsaturated fatty acid; DMAs: Dimethylacteals.

^a Include some minor components not shown in the table.

^b Includes n-9 and n-7 isomers.

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^d DHA/EPA, 22: 6 n-3/ 20: 5 n-3; EPA/ARA, 20: 5 n-3/ 20:4 n-6.

Figure 1.

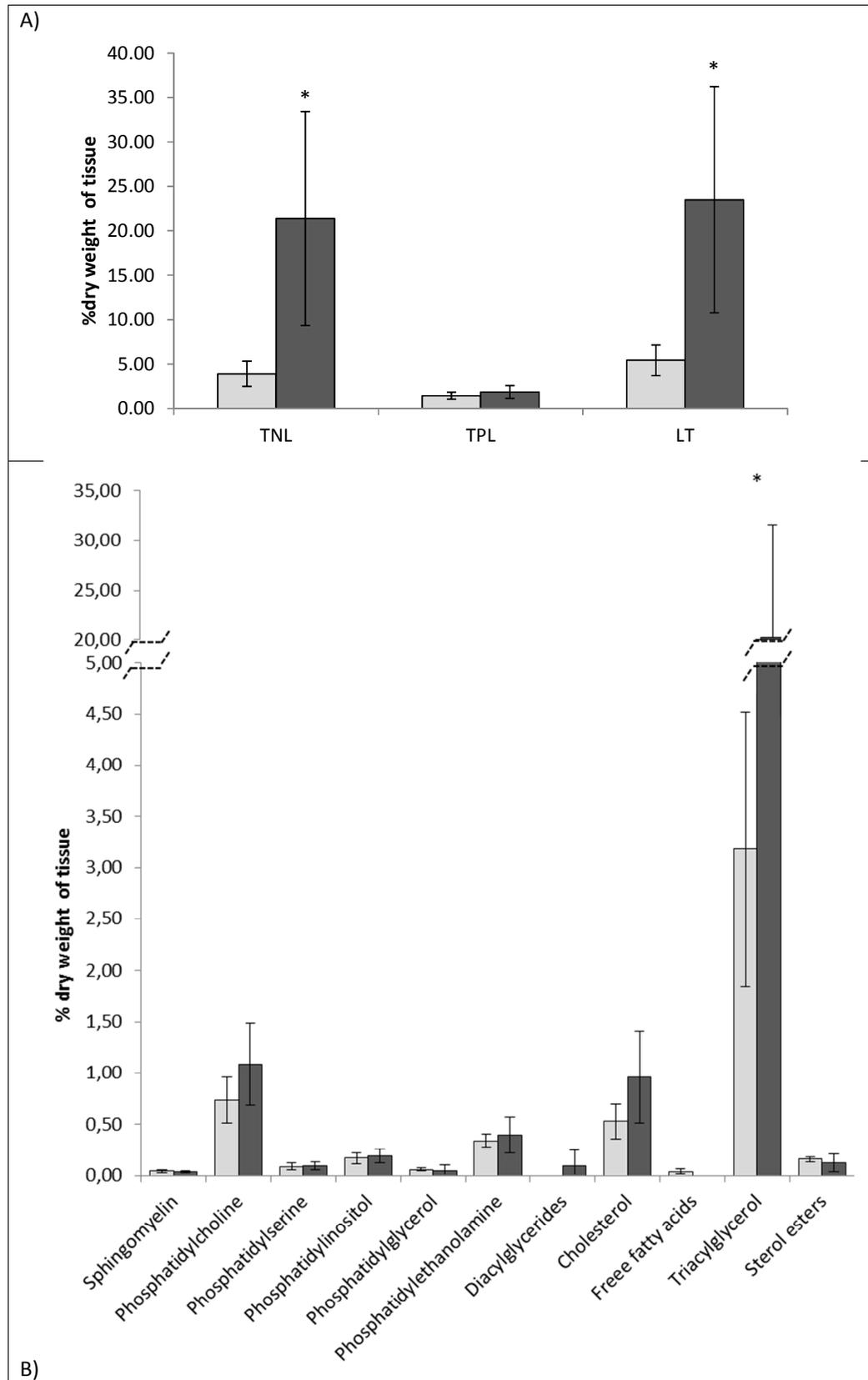


Figure 2.

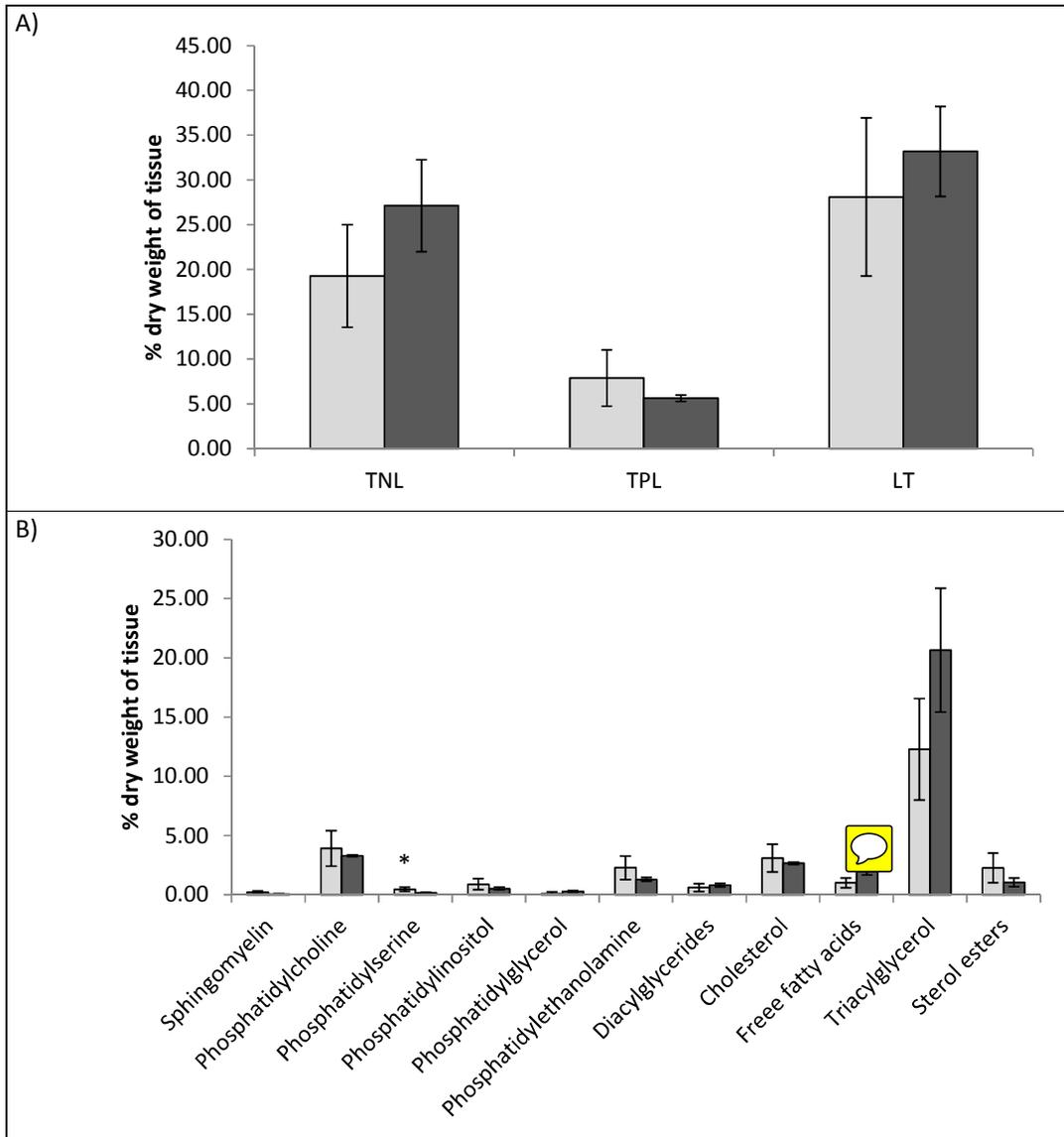


Figure 3.

