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

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

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Running title: Rearing conditions effect on Seriola lipid profile

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Abstract

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

Introduction

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

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

gonad development, or the maintenance of eggs production at the expense of somatic tissue the two possible strategies adopted by most of the species. Therefore, culture conditions have a direct effect on the pattern of utilization and mobilization of energy reserves (Portz et al., 2006). Thus, depletion in lipid reserves or some specific fatty acids has been observed in several species kept under high stocking densities (Papautsoglou *et al.,* 2006; Karakatsouli *et al.,* 2007; Montero *et al.,* 1999, 2001). Spontaneous spawning under captivity usually needs moderate to large holding volumes and low stocking densities in most fish species, being tank size, water column depth or stocking density parameters that have been proved to influence reproductive success in some cultured fish (Mylonas *et al.*, 2010). In fact, despite the difficulties encountered with the reproduction of greater amberjack (Kozul et al., 2001; Lazzari et al., 2000; Mylonas *et al*., 2004), it have been shown that wild greater amberjack broodstock maintained in large volume raceways tanks, can reach sexual maturation and spawn spontaneously for several consecutive years (Jerez el al. 2006; 2007).

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

Material and Methods

Fish and experimental conditions

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

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

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

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

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

Sampling methods

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

Assay methods

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

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

with the Levene test. Differences between pairs of means were tested using Student´s t-test. In all statistical tests used, $p<0.05$ was considered significantly different. Statistical analysis was carried out using the IBM SPSS statistics package (version 20.0 for Windows).

Results

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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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.

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

	Indoor	*Outdoor*	
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.

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:	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.

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 tracylglycerides fraction from cultured *Seriola dumerili* reared in indoor and outdoor conditions.

Fatty acids	Muscle		Liver		Ovary	
	Indoor	*Outdoor*	*Indoor*	*Outdoor*	*Indoor*	*Outdoor*
saturartes[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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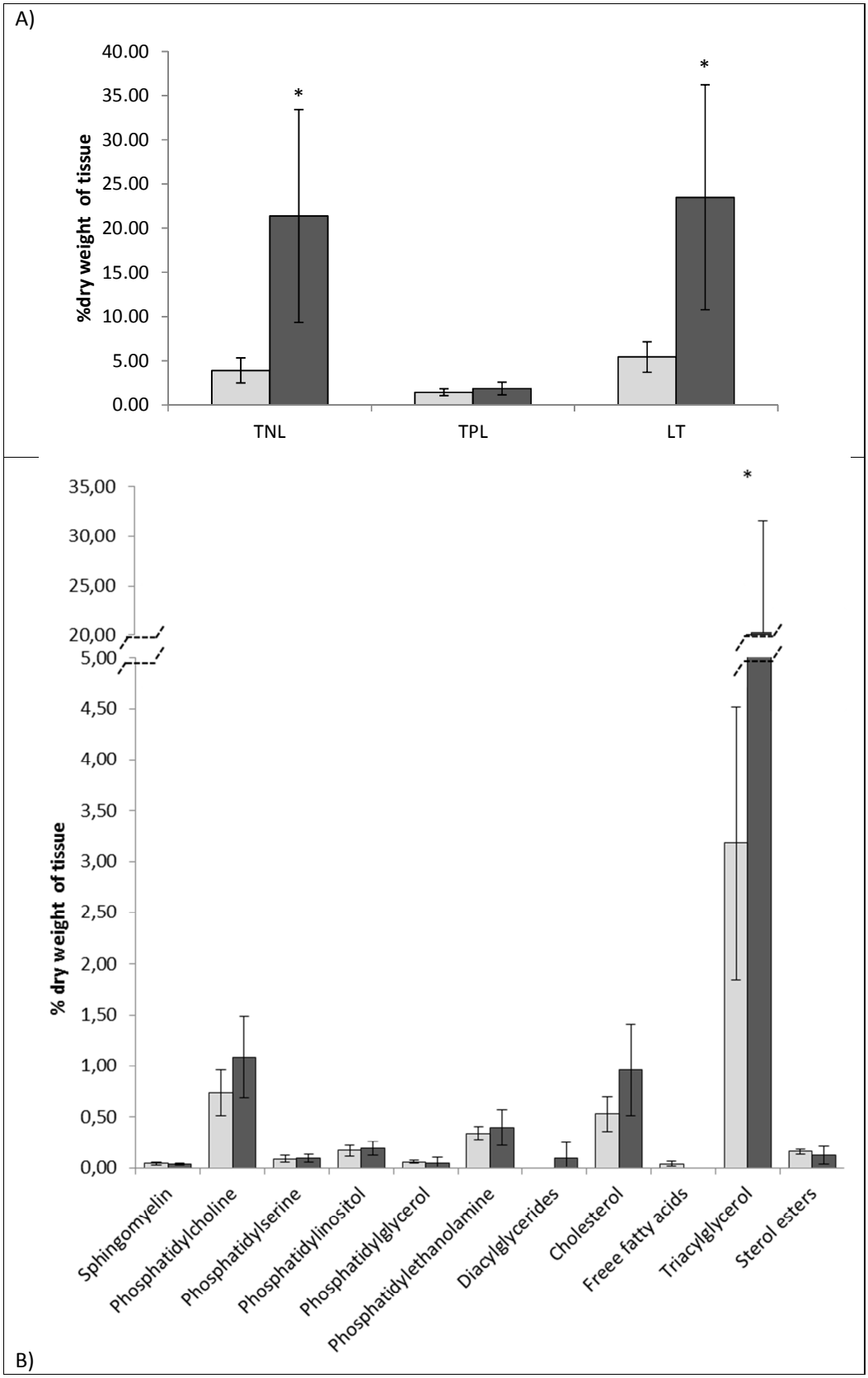
[d] DHA/EPA, 22: 6 *n*-3/ 20: 5 *n*-3; EPA/ARA, 20: 5 *n*-3/ 20:4 *n*-6.

Figure 1.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20.00
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10.00
5.00
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%dry weight of tissue
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TNL
TPL
LT
B)
35,00
30,00
25,00
20,00
5,00
4,50
4,00
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3,00
2,50
2,00
1,50
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0,50
0,00
% dry weight of tissue
*
Sphingomyelin
Phosphatidylcholine
Phosphatidylserine
Phosphatidylinositol
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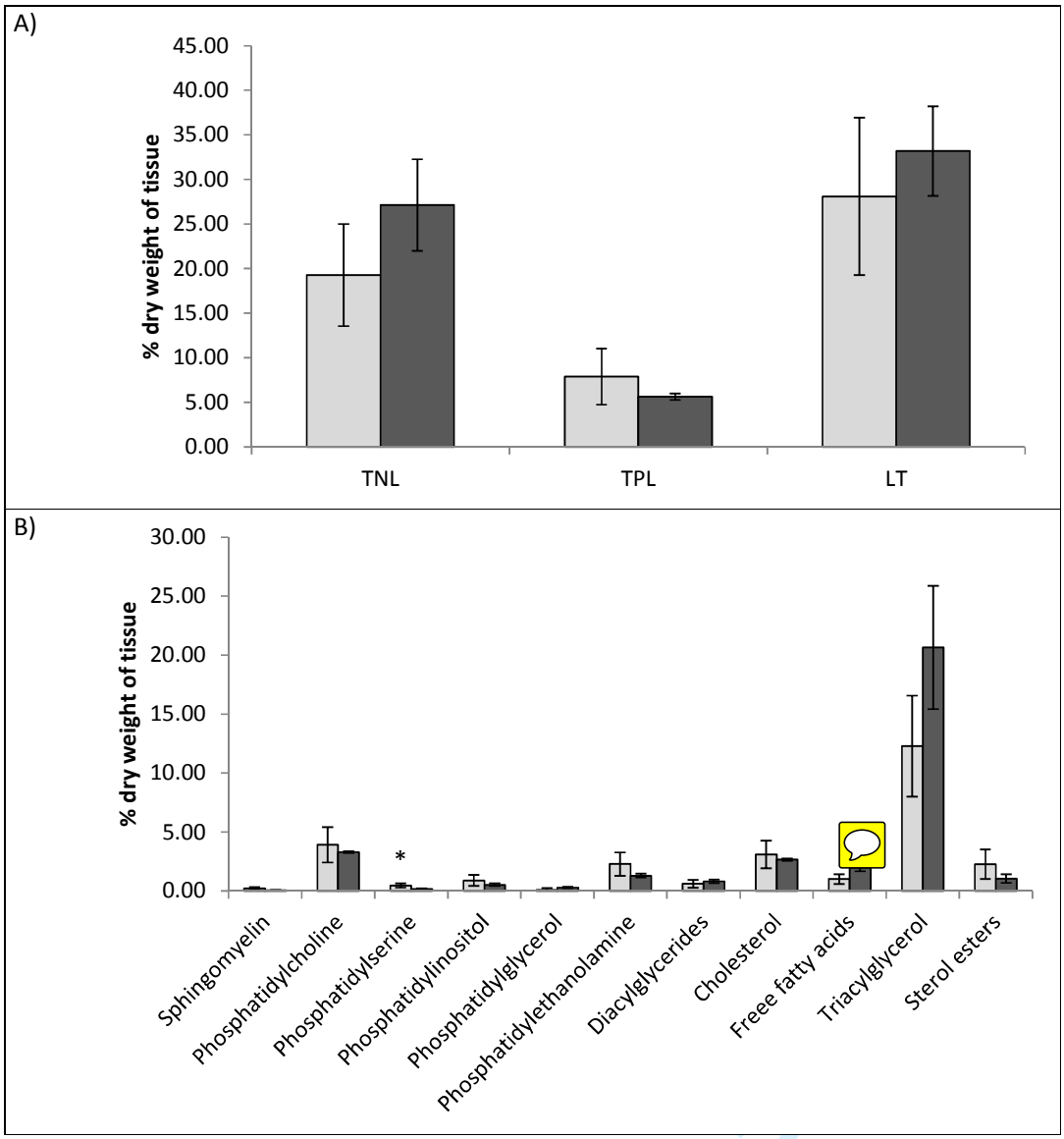
Phosphatidylethanolamine
Diacylglycerides
Cholesterol
Freee fatty acids
Triacylglycerol
Sterol esters

Figure 2.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B)
30.00
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% dry weight of tissue
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Sphingomyelin
Phosphatidylcholine
Phosphatidylserine
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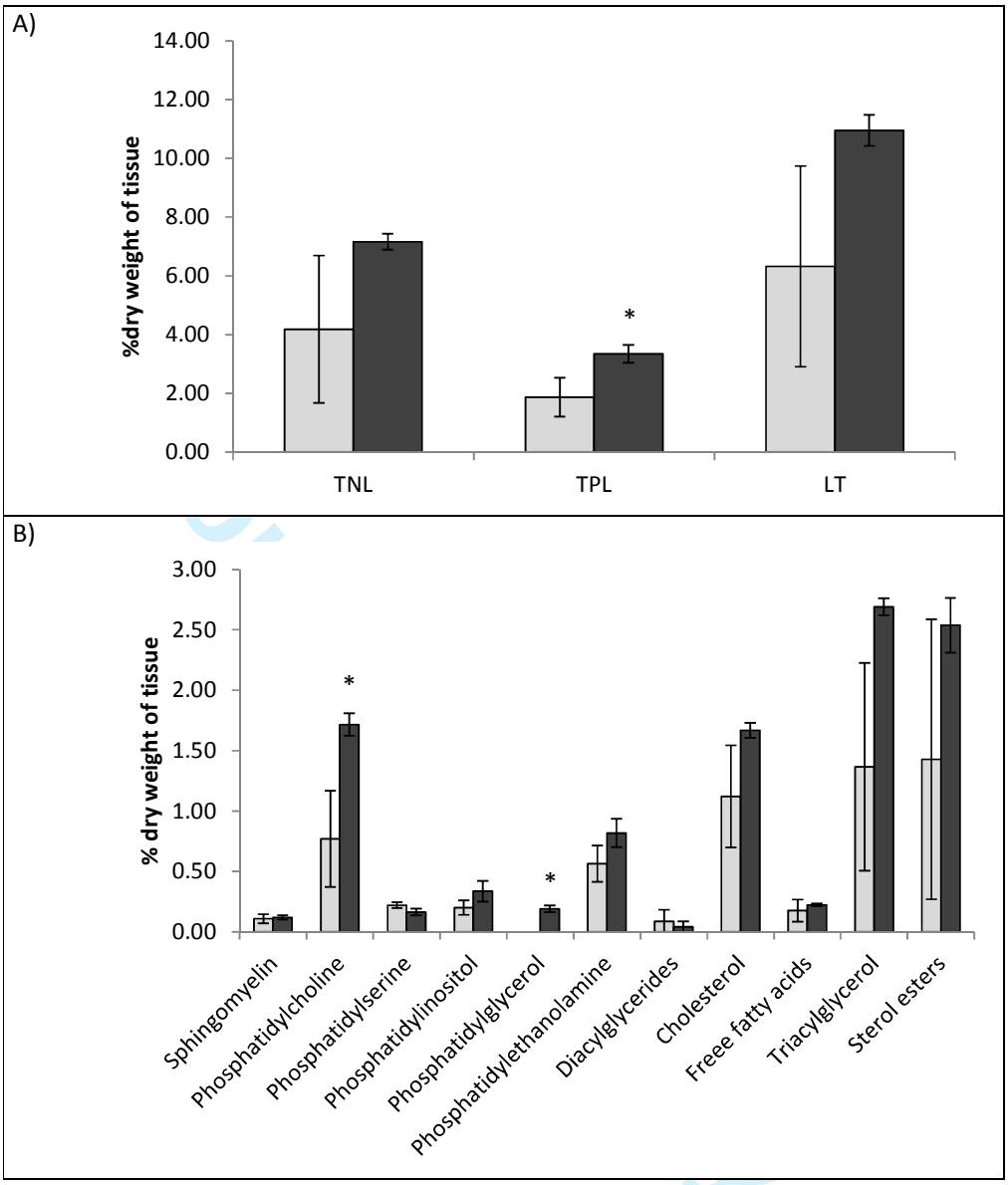
Phosphatidylethanolamine
Diacylglycerides
Cholesterol
Freee fatty acids
Triacylglycerol
Sterol esters

Figure 3.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10.00
8.00
6.00
4.00
2.00
0.00
*
TNL
TPL
LT
B)
% dry weight of tissue
3.00
2.50
2.00
1.50
1.00
0.50
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Sphingomyelin
Phosphatidylcholine
Phosphatidylserine
Phosphatidylinositol
Phosphatidylglycerol
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