Research Article

Ovary and egg fatty acid composition of greater amberjack broodstock (*Seriola dumerili*) fed different dietary fatty acids profiles

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The aim of this study was to assess the effect of an experimental diet (ED), with high levels of 18:1 *n*-9 and low eicosapentaenoic to arachidonic acid ratio (EPA/AA), on the fatty acid (FA) profile of ovary and eggs of *Seriola dumerili* broodstock, in contrast to a non-specific commercial diet (nsCD), taking wild fish lipid composition as a positive reference. Two groups of Seriola broodstock born in captivity were fed with either the ED or the nsCD during two consecutive spawning seasons (21 months). After 7 months of feeding, fish fed the ED displayed an ovary FA profile much closer to wild fish. During the second spawning season, only the group fed ED released eggs. Egg FA composition showed some minor changes throughout the spawning season, with a marginal reduction of EPA in the late season being the most striking variation. Overall, the use of the ED showed some positive results, which could favor spontaneous egg release from females born in captivity. However, the lack of fertilization and the high level of 18:2 *n*-6 in the ovary tissue and eggs indicate that further improvements are needed in *S. dumerili* broodstock diet formulation in order to enhance the reproductive performance of this species in captivity.

Practical applications: The use of the ED resulted in an ovary fatty acid profile of cultured females that better resembles that of wild fish. Using broodstock diets with balanced EPA/AA ratios (close to wild fish) may have a positive effect on fish broodstock reproductive performance, at least for this species.

Keywords: Broodstock diet / Eggs / Fatty acids / Ovary / *Seriola dumerili*

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1 Introduction

Greater amberjack (*Seriola dumerili*, Risso 1810) is a species with great potential for the global aquaculture industry. It is one of the most relevant farmed species in Japan [1, 2] and is considered a leading candidate for the marine aquaculture diversification in the Mediterranean region [3]. This epipelagic carnivorous species, distributed worldwide in temperate and tropical waters, has an excellent flesh quality, a high market price, and a high growth rate in wild and captivity conditions [4–10]. Despite this wide range of features, the difficulties found for its reproduction in captivity

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Abbreviations: ED, experimental diet; **GSI,** gonadosomatic index; **HUFA,** highly unsaturated fatty acids; **LC,** lipid class; **nsCD,** non-specific commercial diet; **PC,** phosphatidylcholine; **PCA,** principal component analysis; **PC1,** principal component 1; **PE,** phosphatidylethanolamine; **RO,** rapeseed oil; **TG,** triacylglycerides; **TL,** total lipid

has hampered the commercial culture of this species, with it being limited to the fattening of wild-caught juveniles [11]. Hormone-induced [12, 13] and spontaneous spawning [6] have been achieved from wild fish kept in captivity for different periods of time, however, the results obtained for fertility and egg quality are fairly limited and need to be improved to support a stable supply of fingerlings. Although, reproduction success in captivity has been reached from greater amberjack broodstock fed raw fish [6, 12, 13]; no spawn have been reported from fish fed either commercial or experimental dry pellets. Published research on this species is currently lacking information on several relevant aspects about broodstock nutrition which may affect its reproductive performance. Regarding nutrients, lipids, fatty acids, and specifically highly unsaturated fatty acids (HUFA) play an important role in the reproductive processes. In particular, DHA (22:6 *n*-3), EPA (20:5 *n*-3), and AA (20:4 *n*-6) are EFA for most marine teleost [14, 15] and several studies have shown their role in the regulation of reproduction in many fish species [16–21]. Supplying a proper level of EFA on broodstock diet is vital to produce eggs with the suitable contents of these fatty acids to ensure embryo and larvae development [15, 17, 22]. On the other hand, EPA and AA are precursors of eicosanoids, a group of active compounds with very important physiological functions such as reproduction, immune response, renal function, etc. [23]. AA derived eicosanoids have shown to be involved in pheromonal attraction, steroidogenesis, and oocytes maturation [24–28]. Thus, the proportion of these two fatty acids in broodstock diets is not only important for its relevance to the vitellum composition, but also because of its very role in the regulation of reproductive physiology [16, 19].

Bearing in mind that the fatty acid profile of tissue is clearly influenced by diet [29–31] and that comparisons between wild and cultured specimens have proved to be a reliable assessment of diet suitability for lipid nutrition [29, 32–35], previously, a work on lipid and fatty acid composition of different tissues from wild and cultured mature females of greater amberjack [36] was carried out. The study showed that tissues from cultured mature females fed a commercial non-specific diet displayed lower levels of 18:1 *n*-9, much higher levels of EPA and consequently higher EPA/AA ratios, than their wild counterparts. Those results suggested that some fatty acids, especially 18:1 *n*-9, EPA and AA, were not provided in the appropriate proportions in the diet supplied to the cultured stock and this could be negatively affecting their reproductive performance.

Based on the fatty acid profile of wild specimens, and on the deficiencies observed in cultured fish [36], an experimental diet with a more suitable fatty acid profile was designed to be tested in the present study.

The aim of this research, that have been carried out with greater amberjack broodstock born in captivity, was to determine the effect of the experimental diet on the fatty acid profile of ovary and eggs of greater amberjack, in contrast to a non-specific commercial diet (nsCD), taking wild fish lipid composition as a positive reference.

2 Material and methods

2.1 Fish and experimental conditions

A broodstock group of greater amberjack (*S. dumerili*) (6 years old), born in captivity in the experimental culture facilities of the Spanish Institute of Oceanography (Tenerife, Canary Islands, Spain), was used to carry out this study. In order to keep the fish in the conditions under which natural spawning of this species has been previously achieved [6], only two groups of fish could be formed due to the number of large-volume tanks and specimens available. In February 2011, 40 fish were randomly distributed in two outdoor 500 m^3 raceway tanks (20 fish per group, with 9.51 ± 2.86 and 11.13 ± 4.11 kg average weight, and sex ratio close to 1:1) with open flow-through seawater system, continuous water supply adjusted to 6 renewals tank day-1, ambient water temperature (18.8–24.1°C), and natural photoperiod with sunlight intensity attenuated by tank covers. One group of fish was fed with a nsCD (Turbot diet R22, Skretting, Spain), and the other group was fed with an experimental diet (ED) formulated to contain the same amount of protein and lipid that the commercial diet, but with a different fatty acid profile, including higher proportions of 18:1 *n*-9 and a lower EPA/AA ratio, based on our previous studies [36]. The experimental diet was manufactured by SPAROS Lda. (Algarve, Portugal). Fish were hand feed once a day and 3 days a week (1% of biomass day-1).

Both fish groups were kept in the aforementioned experimental conditions for 21 months (February 2011–October 2012), a period that includes two consecutive breeding seasons. During the spawning period, which takes place in similar culture conditions between April and October [6], each tank was fitted with an overflow egg collector and checked daily. For each spawning, the date and number of eggs released were recorded, and eggs were examined under the microscope to check fertilization.

2.2 Fish and egg sampling

In August 2011, during the second half of the first spawning season and after 7 months of being fed with either the nsCD or the ED, five mature females from each group were sacrificed by an anesthetic overdose (2-phenoxiethanol, 600 ppm). At the same time, five mature female (14.2 ± 5.8 kg average weight) were captured from the wild. For each female, biometric parameters of length, body weight, and gonad weight were registered, gonadal maturity was confirmed by visual examination [37] and gonadosomatic index (GSI) was calculated using the following formula: $GSI = 100$ (Ovary wt $\times$ body wt^{-1}). Samples of ovary tissue were collected and

stored at −80°C for lipid analysis. During the first breeding season, egg samples could not be collected, since none spawning were obtained by either group. In the course of the second spawning season, eggs were released from April till October 2012, and samples of eggs (three in Early-season, three Mid-season, and three in Late-season) were collected and preserved for lipid analyses.

2.3 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 [38].

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.[39]. The organic solvent was evaporated under a stream of nitrogen and the lipid content was determined gravimetrically [40] 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 TLC (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 classes were visualized by charring with 3% w/v aqueous cupric acetate containing 8% v/v phosphoric acid, and quantified by scanning densitometry using a dual-wavelength flying spot scanner Shimadzu CS-9001PC (Shimadzu, Duisburg, Germany) [41]. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and triacylglycerides (TG) were purified by TLC using the polar solvent system described before for PC and PE purification, and the neutral solvent system for TG. The separated classes were sprayed with 0.1% 2′,7′-diclorofluorescein in methanol (98%) w/v, containing BHT, and visualized under ultraviolet light. Bands were scraped off the plates into tubes for the subsequent analysis of fatty acids.

To determine the fatty acid profiles, TL extracts and PC, PE, and TG fractions were subjected to acid-catalyzed transmethylation with 1% sulfuric acid v/v in methanol. The resultant FAME were purified by TLC [40]. During acid-catalyzed transmethylation, FAME are formed simultaneously with dimethyl acetals (DMAs) which originate from the 1-alkenyl chain of plasmalogens. FAME and DMA were separated and quantified using a TRACE-GC Ultra gas chromatograph (Thermo Scientific) equipped with an on-column injector, a FID and a fused silica capillary column, Supelcowax TM 10 (Sigma–Aldrich, Madrid, Spain). Individual FAME and DMA were identified by reference to authentic standards, and further confirmation of FAME and DMAs identity was carried out by GC–MS (DSQ II, Thermo Scientific).

2.4 Statistical analysis

Results are reported as means ± SD. Non-detected fatty acids were considered as 0 value for statistical analysis. Arcsin transformation was performed for proportion data. Normal distribution was checked for all data with the one-sample Kolmogorov–Smirnoff test and homogeneity of the variances with the Levene test. Comparisons between groups (Ovary: nsCD, ED, Wild/Eggs: Early-season, Mid-season, Late-season) were assessed by one-way ANOVA followed by the Tuckey post hoc test. When homoscedasticity and/or normality was not achieved, data were subjected to Kruskal–Wallis test (non-parametric test) followed by Games–Howell post hoc test. Fatty acids were additionally submitted to Principal components Analysis (Factor analyses), and factor scores were subsequently analyzed by one-way ANOVA. 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).

3 Results

3.1 Diets

The composition of the diets used to feed cultured *S. dumerili* broodstock groups in this experiment is shown in Tables 1 and 2. Both diets contain approximately the same amount of protein and lipid (% of dry weight), but different fatty acid profiles which reflects the different proportions of fish oil and vegetable oil used in their manufacture (nsCD: 13% fish oil; ED: 2.1% fish oil, 8.2% rapeseed oil (RO), and 1.70% Algaritum DHA70). Although there are many differences in the fatty acid composition between the two diets tested, noteworthy was the higher content of 18:1 *n*-9, 18:2 *n*-6, and 18:3 *n*-3 in the experimental diet as a result of using RO in their production, as well as the marked reduction of EPA in ED respect to nsCD, which results in a considerable reduction of the EPA/AA ratio and increases of the DHA/EPA ratio in the experimental diet.

Table 1. Proximate composition (% dry weight) of the non-specific diet (nsCD) and the experimental diet (ED) supplied to *Seriola dumerili* broodstocks

	nsCD	ED
Proximate composition (% d.w.)		
Crude protein	54.00	52.12
Crude lipid	20.20	21.20
Crude fiber	1.50	1.31
Carbohydrates	12.13	13.78
Total phosphorus	1.80	1.80
Ash	10.37	9.79

Table 2. Moisture (%), total lipid (% dry weight), and main fatty acid composition (% total fatty acids) of the non-specific diet (nsCD) and the experimental diet (ED) supplied to *Seriola dumerili* broodstocks

	nsCD	ED
Moisture	12.49 ± 0.12	12.04 ± 0.63
Total lipid	20.20 ± 1.16	21.20 ± 0.28
Main fatty acids		
14:0	6.42 ± 0.02	2.57 ± 0.08
16:0	19.71 ± 0.11	11.58 ± 0.10
16:1[a)]	8.23 ± 0.07	2.77 ± 0.08
18:0	3.90 ± 0.01	2.76 ± 0.04
18:1 *n*-9	10.58 ± 0.04	34.36 ± 0.08
18:1 *n*-7	2.92 ± 0.01	3.28 ± 0.02
18:2 *n*-6	5.91 ± 0.37	12.40 ± 0.01
18:3 *n*-3	0.89 ± 0.02	4.60 ± 0.04
18:4 *n*-3	2.14 ± 0.03	0.61 ± 0.01
20:1[b)]	0.86 ± 0.01	2.31 ± 0.01
20:4 *n*-6	1.00 ± 0.00	0.46 ± 0.00
20:5 *n*-3	14.60 ± 0.20	4.17 ± 0.07
22:1[b)]	0.54 ± 0.05	1.95 ± 0.15
22:5 *n*-6	0.32 ± 0.02	0.45 ± 0.02
22:5 *n*-3	1.80 ± 0.01	0.98 ± 0.02
22:6 *n*-3	11.37 ± 0.16	10.32 ± 0.42
∑ Saturates	31.44 ± 0.06	18.56 ± 0.25
∑ Monoenes	24.00 ± 0.07	45.26 ± 0.19
∑ PUFA	44.01 ± 0.12	35.61 ± 0.12
∑ *n*-3 HUFA	29.02 ± 0.22	16.01 ± 0.22
∑ *n*-6 HUFA	1.43 ± 0.17	1.34 ± 0.17
DHA/EPA	0.78 ± 0.02	2.47 ± 0.02
EPA/AA	14.57 ± 0.18	9.00 ± 0.20

Results are expressed as means ± SD (two replicates).
[a)]Includes *n*-9 and *n*-7 isomers.
[b)]Includes *n*-11, *n*-9, and *n*-7 isomers. tr, values ≤0.20%.

3.2 Ovaries

Cultured fish fed with either nsCD or ED did not show significant differences between them in terms of body size or body weight, and fish fed ED neither showed significant differences from wild fish for those parameters (Table 3). Mature females sacrificed after 7 months of being fed with the commercial or the experimental diet displayed similar GSI values (1.12 ± 0.20 and 0.92 ± 0.38 for nsCD and ED groups, respectively), with both of them being significantly lower than GSI of wild broodstock (3.43 ± 0.84) ($p < 0.05$) (Table 3). TL content, neutral and polar lipid relative proportions and fatty acid composition of ovary from cultured (nsCD and ED) and wild fish are shown in Table 4. Both groups of cultured fish displayed lower TL content than wild fish, with ED group bearing the lowest value. Despite these differences in the lipid content, no differences in the relative proportions of total neutral lipid (TNL) and total polar lipid (TPL) were found between ED and wild fish, with both being significantly different to those values found in nsCD specimens.

Table 3. Mean size (cm), mean weight (kg), and gonadosomatic index (IGS) of wild fish and cultured fish fed on the non-specific commercial diet (nsCD) and the experimental diet (ED)

	nsCD	ED	Wild	
Size	69.40 ± 8.88[b]	79.40 ± 8.67[ab]	99.96 ± 17.07[a]	P
Weight	7.52 ± 2.02[b]	8.49 ± 3.26[ab]	14.06 ± 5.11[a]	P
GSI	1.12 ± 0.20[b]	0.92 ± 0.38[b]	3.43 ± 0.84[a]	NP

Results are expressed as means ± SD ($n = 5$ replicates). Values in the same row bearing different superscript letter (a–c) show significant differences ($p \leq 0.05$), (a) was always assigned to the wild group.

Regarding fatty acids composition of ovary total lipid, the ED group showed a profile closer to wild fish than nsCD group (Table 4 and Figure 1). No differences among groups were found in the most abundant saturates such as 16:0 or 18:0, but some minor differences were found for short chain saturates such as 14:0 and 15:0. Ovary from ED group presented 18:1 *n*-9 proportions with values in between wild and nsCD groups, without being significantly different of any of them. Among the *n*-6 fatty acids, all groups displayed different levels of 18:2 *n*-6 with ED showing the higher proportion, while AA and 22:5 *n*-6 exhibited higher proportions in ED group and wild fish than in nsCD group. ED and wild animals did not showed significant differences between them for these *n*-6 HUFA. With respect to *n*-3 fatty acids, no differences were found in EPA proportions between ED and wild fish, with both showing considerably lower proportions than animals fed with nsCD. No differences in the DHA level were found among groups. It is worth noting that wild fish and fish fed with ED did not show differences in the EPA/AA ratio, which was substantially lower than that observed in fish fed with the nsCD. The DHA/EPA ratio was different among groups, and ED group displayed a DHA/EPA ratio with values in between wild and nsCD groups.

Principal component analysis (PCA) of total lipid fatty acid profile showed the proximity of ovary composition between wild and ED fish (Figure 1). Thus, the two components of PCA accounted for the 67.71% of variation of this data set. The principal component 1 (PC1) explained 38.85% of variation, and it clearly separated fatty acids that were considered as specially relevant to the design of the experimental diet, such as 18:1 *n*-9 (on the left) and EPA (on the right) (Figure 1A). ED and wild fish were clustered together and were significantly separated ($p < 0.05$) from the group fed the nsCD when considering factor score 1 (Figure 1C). The principal component 2 (PC2) accounted for 28.88% of variation, and it separated fatty acids that come from vegetable oils such as 18:2 *n*-6, and 18:3 *n*-3, more abundant in ED (above the zero line), from those, such as 16:1, more abundant in nsCD (below the zero line) (Figure 1A). The three groups were significantly separated ($p < 0.05$) when considering factor score 2 (Figure 1C).

Table 4. Total lipid content (% dry weight), total neutral lipid (TNL) and total polar lipid (TPL) proportions (%), and fatty acid composition (% total fatty acids) of ovary total lipid extract from cultured specimens of *Seriola dumerili* fed on the non-specific diet (nsCD) and the experimental diet (ED), and from wild specimens

	nsCD	ED	Wild	
Total lipid	12.30 ± 3.83^{b}	5.51 ± 3.32^{c}	17.01 ± 1.36^{a}	NP
TNL	74.64 ± 1.06^{b}	62.52 ± 5.71^{a}	66.94 ± 6.74^{a}	NP
TPL	24.55 ± 1.41^{b}	34.14 ± 7.44^{a}	32.21 ± 6.62^{a}	NP
Fatty acids				
14:0	1.58 ± 0.27^{a}	0.83 ± 0.16^{b}	1.03 ± 0.45^{ab}	P
15:0	0.25 ± 0.03^{b}	0.22 ± 0.03^{b}	0.43 ± 0.10^{a}	P
16:0	17.89 ± 1.19	17.44 ± 0.47	17.94 ± 1.11	P
16:1[a)]	4.13 ± 0.75^{a}	1.74 ± 0.35^{b}	3.93 ± 0.45^{a}	P
16:2 (*n*-3)	0.45 ± 0.10^{b}	0.34 ± 0.03^{b}	1.08 ± 0.23^{a}	P
17:0	0.57 ± 0.08	0.73 ± 0.10	0.74 ± 0.15	P
16:3 (*n*-4)	0.22 ± 0.03^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	NP
16:3 (*n*-3)	0.30 ± 0.02^{b}	0.22 ± 0.03	0.77 ± 0.08^{a}	P
18:0	5.32 ± 0.88	5.48 ± 0.97	4.50 ± 0.98	P
18:1(*n*-9)	14.89 ± 1.08^{b}	18.87 ± 2.35^{ab}	24.92 ± 4.75^{a}	P
18:1 (*n*-7)	3.74 ± 0.32	3.96 ± 0.19	3.84 ± 0.42	P
18:2 (*n*-6)	4.44 ± 0.85^{b}	7.54 ± 1.73^{c}	1.39 ± 0.12^{a}	P
18:3 (*n*-3)	0.42 ± 0.11^{a}	1.07 ± 0.39^{b}	0.34 ± 0.11^{a}	P
18:4 (*n*-3)	0.60 ± 0.14^{b}	0.07 ± 0.08^{a}	0.16 ± 0.11^{a}	P
20:1[b)]	1.17 ± 0.07^{b}	0.85 ± 0.08^{a}	0.78 ± 0.27^{a}	NP
20:4 (*n*-6)	2.96 ± 1.09^{b}	4.55 ± 1.14^{a}	4.11 ± 0.38^{a}	P
20:4 (*n*-3)	0.54 ± 0.09	0.18 ± 0.03	0.42 ± 0.12	P
20:5 (*n*-3)	8.88 ± 1.17^{b}	3.98 ± 0.17^{a}	3.08 ± 0.71^{a}	P
22:1[b)]	0.24 ± 0.08^{b}	0.37 ± 0.02^{b}	0.03 ± 0.06^{a}	P
22:5 (*n*-6)	0.57 ± 0.09^{b}	1.05 ± 0.23^{a}	1.38 ± 0.16^{a}	P
22:5 (*n*-3)	3.25 ± 0.19^{b}	2.03 ± 0.25^{c}	2.85 ± 0.23^{a}	P
22:6 (*n*-3)	23.44 ± 3.07	22.57 ± 1.34	23.34 ± 4.08	P
18:0 DMA[c)]	0.35 ± 0.24^{a}	0.00 ± 0.00^{b}	0.28 ± 0.03^{a}	NP
18:1 (*n*-9) DMA[c)]	0.31 ± 0.43^{ab}	0.88 ± 0.22^{b}	0.03 ± 0.03^{a}	P
18:1 (*n*-7) DMA[c)]	0.05 ± 0.12^{a}	0.82 ± 0.27^{b}	0.00 ± 0.00^{a}	NP
Totals				
$\sum$ Saturartes[d)]	25.64 ± 2.08	25.09 ± 1.37	24.72 ± 0.56	P
$\sum$ Monoenes[d)]	24.52 ± 1.11^{b}	25.92 ± 2.55^{b}	34.66 ± 4.72^{a}	P
$\sum$ PUFA[d)]	47.36 ± 3.41	44.23 ± 0.72	39.33 ± 5.00	P
$\sum$ HUFA[d)]	40.43 ± 2.93	35.09 ± 2.59	36.08 ± 4.25	P
Ratios				
EPA/AA[e)]	3.32 ± 1.07^{b}	0.93 ± 0.28^{a}	0.74 ± 0.22^{a}	P
DHA/EPA[e)]	2.65 ± 0.26^{c}	5.67 ± 0.32^{b}	8.03 ± 1.32^{a}	P

Results are expressed as means ± SD ($n = 5$ replicates). Some minor components with trace values (≤0.20%) are not included in the table. Values in the same row bearing different superscript letter (a–c) show significant differences ($p \leq 0.05$), (a) was always assigned to the wild group. *P* = parametric tests, one-way ANOVA followed by Tukey post hoc; *NP* = non-parametric test, Kruskal–Wallis followed by Games–Howell post hoc.

[a)] Includes *n*-9 and *n*-7 isomers.

[b)] Includes *n*-11, *n*-9, and *n*-7 isomers.

[c)] DMA = dimethyl acetals.

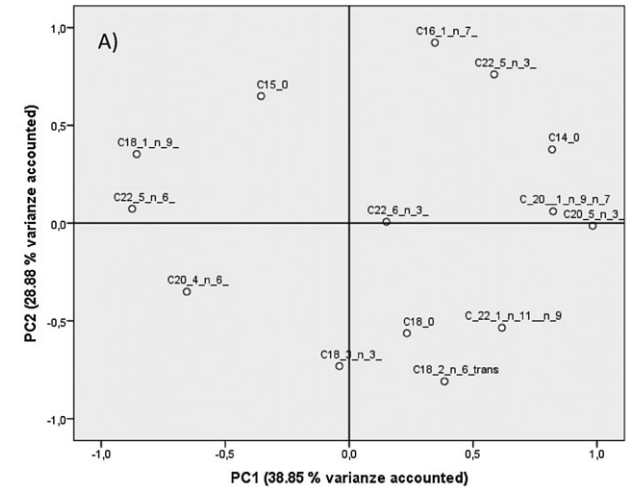
[d)] Includes some minor components not shown in the table.

[e)] DHA/EPA, 22:6 *n*-3/20:5 *n*-3; EPA/AA, 20:5 *n*-3/20:4 *n*-6.

3.3 Eggs

During the first spawning season none of the fish groups, neither the one fed with ED, nor the one fed with the nsCD, released eggs. During the second spawning season, only the group fed with ED spawn spontaneously, releasing a total of 13 eggs batches from April till September, albeit were unfertilized eggs.

Over the period of spawning, no differences were found in TL, TPL, TNL content (Table 5) and in the lipid class profile



B)

	nsCD	*ED*	*Wild*
PC1	b	a	a
PC2	c	b	a

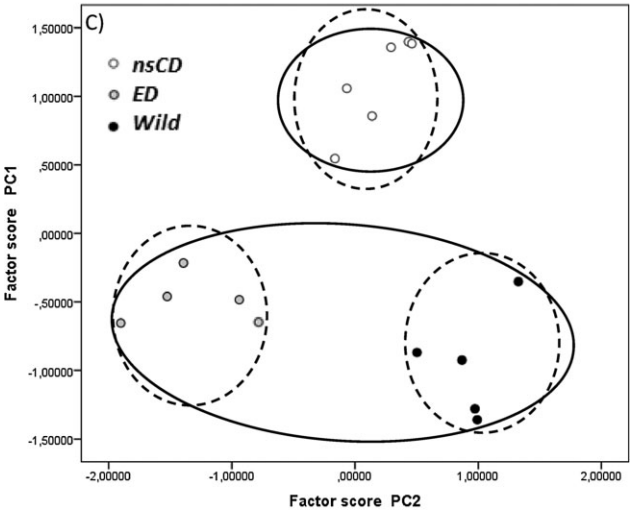


Figure 1. Principal component analysis (PCA) of total lipid fatty acids from ovary of fish fed nsCD, ED, and wild fishes. (A) Component loading plot for the PCA (graphical representation of the correlation between each specific variable and the principal components PC1 and PC2). (B) Results obtained by submitting factor scores to ANOVA test. Different superscript letter (a–c) show significant differences ($p \leq 0.05$) among groups for the factor scores (PC1 and PC2). (C) Factor score plot (graphical representation of individual scores for each PC). Bold line ellipse represents different clusters for PC1 according ANOVA results; Dash line ellipse represents different clusters for PC2 according ANOVA results. [(◯) nsCD = fish fed non-specific commercial diet; (●) ED = fish fed experimental diet; (●) Wild = wild fish].

(data not shown) of egg batches released by the ED group. The fatty acid profile of TL extract from ED-eggs experienced slight variations along the spawning season. No differences were found between eggs obtained in early-season and mid-season, but both of them displayed marginally lower proportions of 16:0, 16:1, 18:2 *n*-6, and 22:5 *n*-3, and slightly higher proportions of 14:0, 18:0, AA and EPA when compared to eggs obtained in late-season. PCA, used to examine the multivariate structure of the data set, confirmed the differences along the spawning season (Figure 2). Thus, the two components of PCA accounted for the 81.3% of variation of this data set, with the 63.87% of variation being explained by PC1 itself (Figure 2A). For factor score 1, early-season and mid-season eggs were grouped together, and significantly separated from the late-season eggs (Figure 2C). Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and triacylglycerides (TG) fatty acid profile were submitted to PCA and ANOVA for the factor scores (Figure 3), and only significant differences were found in the TG fatty acid profile (Figure 3D–G). Thus, the differences in the total lipid fatty acid profile in late-season eggs were mainly due to variations in the TG fatty acid profile (Figure 3G; Supplementary Table S3), although some differences for individual fatty acids were also found in PC fatty acid profile (Table S2). For the TG fatty acids data set, both PCA components accounted for the 75.61% of variation, with 44.52% variance accounted by PC1 (Figure 3C). The PC1 separated 16 C and 22 C *n*-3 fatty acids (on the left) and 18 C and 20 C fatty acids (on the right), with 16:0, 18:4 *n*-3, EPA, and 18:1 *n*-9 having the highest loading factors (Figure 3C). For all the lipid classes analyzed (PC, PE, and TG), the levels of EPA in the late season were significantly lower than those in early and mid-season (Tables S1–S3).

4 Discussion

4.1 Diets

The experimental diet used in the present study was formulated based on previous results [36] to contain a more suitable fatty acid profile than that of the commercial non-specific diet usually supplied to the cultured stock. Different oils were used to manufacture the experimental diet, a diet with higher proportion of 18:1 *n*-9 and lower EPA/AA ratio than the commercial diet, but although this aim was achieved, the use of RO produced a parallel undesirable increase of linoleic acid (18:2 *n*-6). High levels of linoleic acid in commercial diets and consequently in cultured fish tissues have been reported in several cultured species as a result of using not only marine sources but also vegetable sources as ingredients in commercial diets [42]. In the present study both diets supplied to cultured fish showed high levels of linoleic acid, but ED exhibited the highest proportion of this fatty acid, what was reflected in the higher levels of 18:2 *n*-6

Table 5. Total lipid content (% dry weight), total neutral lipid (TNL) and total polar lipid (TPL) proportion (%), and fatty acid composition (% total fatty acids) of non-fertilized eggs total lipid obtained from cultured specimens of *Seriola dumerili* fed on the experimental diet (ED) along the spawning season

	Early season	Mid season	Late season	
Total lipid	25.69 ± 3.89	23.08 ± 5.43	27.66 ± 2.75	P
TNL	73.61 ± 0.58	68.85 ± 4.34	67.55 ± 5.65	P
TPL	26.39 ± 0.58	31.15 ± 4.34	32.45 ± 5.65	P
Fatty acids				
14:0	1.14 ± 0.03^{a}	1.19 ± 0.05^{a}	1.03 ± 0.01^{b}	P
15:0	0.20 ± 0.01	0.20 ± 0.01	0.23 ± 0.01	P
16:0	13.00 ± 0.17^{b}	13.14 ± 0.42^{b}	14.67 ± 0.05^{a}	NP
16:1[a)]	2.50 ± 0.05^{b}	2.62 ± 0.06^{b}	2.93 ± 0.07^{a}	P
17:0	0.34 ± 0.01	0.33 ± 0.00	0.33 ± 0.00	NP
18:0	3.83 ± 0.25^{a}	3.89 ± 0.21^{a}	3.31 ± 0.04^{b}	P
18:1 (*n*-9)	31.11 ± 0.56	31.41 ± 0.38	30.66 ± 0.23	P
18:1 (*n*-7)	3.59 ± 0.09	3.79 ± 0.14	3.69 ± 0.01	P
18:2 (*n*-6)	12.99 ± 0.45^{b}	12.64 ± 0.49^{ab}	13.64 ± 0.12^{b}	P
18:3 (*n*-3)	3.59 ± 0.04	3.51 ± 0.11	2.24 ± 0.22	P
18:4 (*n*-3)	0.24 ± 0.01^{a}	0.26 ± 0.01^{a}	0.13 ± 0.01^{b}	P
20:1[b)]	0.81 ± 0.06^{a}	0.82 ± 0.03^{a}	0.67 ± 0.02^{b}	P
20:4 (*n*-6)	0.97 ± 0.03^{a}	0.95 ± 0.04^{ab}	0.89 ± 0.02^{b}	P
20:4 (*n*-3)	0.22 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	P
20:5 (*n*-3)	3.66 ± 0.21^{a}	3.56 ± 0.11^{b}	2.25 ± 0.03^{b}	P
22:5 (*n*-3)	1.29 ± 0.06^{b}	1.27 ± 0.04^{b}	1.65 ± 0.06^{a}	P
22:6 (*n*-3)	18.45 ± 1.03	18.07 ± 0.63	19.49 ± 0.32	P
18:0 DMA[c)]	0.24 ± 0.00	0.24 ± 0.05	0.28 ± 0.02	P
Totals				
$\sum$ Saturartes[d)]	18.57 ± 0.37	18.79 ± 0.68	19.57 ± 0.07	NP
$\sum$ Monoenes[d)]	38.30 ± 0.69	38.96 ± 0.58	38.24 ± 0.23	P
$\sum$ PUFA[d)]	38.53 ± 0.88	37.72 ± 1.34	38.92 ± 0.44	P
$\sum$ HUFA[d)]	25.09 ± 1.34	24.60 ± 0.86	24.93 ± 0.43	P
Ratios				
EPA/AA[e)]	3.77 ± 0.12^{a}	3.74 ± 0.02^{a}	2.54 ± 0.04^{b}	P
DHA/EPA[e)]	5.05 ± 0.09^{b}	5.08 ± 0.07^{b}	8.66 ± 0.10^{a}	P

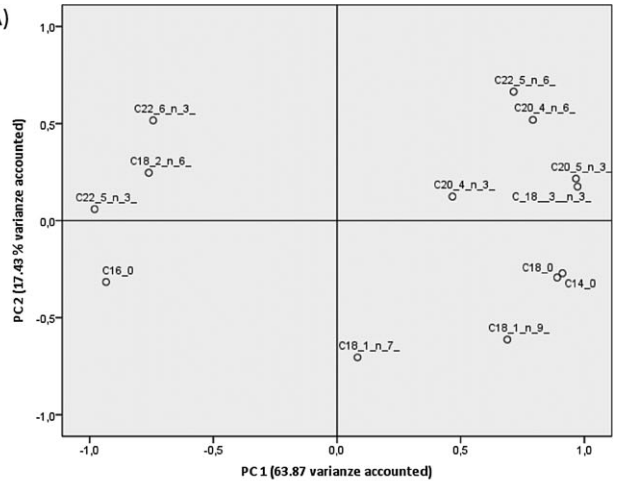
Results are expressed as means $\pm$ SD ($n = 3$ replicates). Some minor components with trace value ($\leq 0.20\%$) are not included in the table. Values in the same row bearing different superscript letter (a and b) show significant differences ($p \leq 0.05$). *P* = parametric tests, one-way ANOVA followed by Tukey post hoc; *NP* = non-parametric test, Kruskal–Wallis followed by Games–Howell post hoc.
[a)] Includes *n*-9 and *n*-7 isomers.
[b)] Includes *n*-11, *n*-9, and *n*-7 isomers.
[c)] DMA = dimethyl acetals.
[d)] Includes some minor components not shown in the table.
[e)] DHA/EPA, 22:6 *n*-3/20:5 *n*-3; EPA/AA, 20:5 *n*-3/20:4 *n*-6.

found in ovary tissue from broodstock fed ED. Considering that 18:2 *n*-6 may be responsible of detrimental modifications on the fatty acid composition of farmed fish [43, 44], it should be reduced to minimum levels in further experimental broodstock diets.

4.2 Ovaries

The analysis of the fatty acid profile of ovaries from females fed for 7 months either the nsCD or the ED revealed that ovaries from ED fish were much closer to wild fish than that from fish fed on nsCD, especially for those fatty acids that were considered more relevant to the design of the experimental diet: 18:1 *n*-9, AA and EPA (Table 4 and Figure 1). The EPA/AA ratio in fish fed with commercial diet was considerably higher than in wild fish, as has been previously observed in other studies in greater amberjack [36] and other species [20, 29, 33–35, 45]. In contrast fish fed with the experimental diet did not display significant differences for the EPA/AA ratio when compared to wild fish. This fact is particularly relevant in terms of oocyte maturation and ovulation, since EPA/AA ratio determine prostaglandins production and the balance between two-series prostanoids, involved in several aspects of the reproductive process such as

A)



B)

	E-season	*M-season*	*L-season*
PC1	a	a	b
PC2	_	_	_

C)

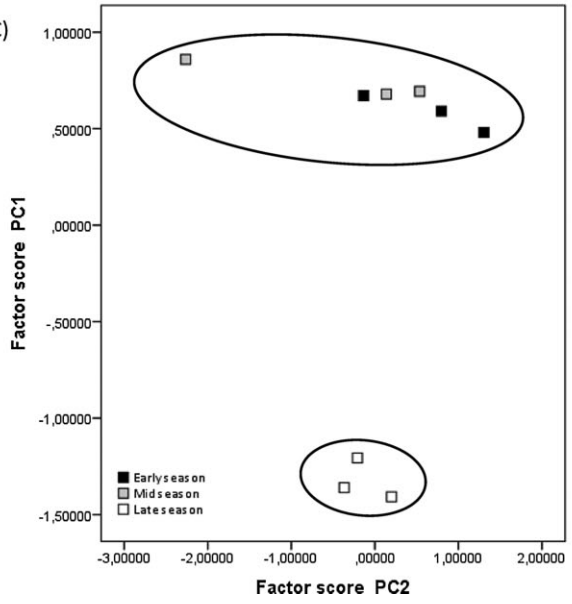


Figure 2. Principal component analysis (PCA) of total lipid fatty acids from non-fertilized eggs obtained from cultured specimens of *Seriola dumerili* fed on the experimental diet (ED) along the spawning season [(■) Early-season; (■) Mid-season; (□) Late season]. (A) Component loading plot (graphical representation of the correlation between each specific variable and the principal components PC1 and PC2). (B) Results obtained by submitting factor scores to ANOVA test. Different superscript letter (a and b) show significant differences ($p \leq 0.05$) among groups for the factor scores. (C) Factor score plot (graphical representation of individual scores for each PC). Bold line ellipse represents different clusters according to ANOVA results.

pheromonal attraction [28], steroidogenesis [24, 25, 46], steroid transport [47], or ovulation and oocyte maturation [26, 27, 48], and three-series prostanoids, which have other biological and physiological targets. Furthermore, several studies have shown the importance of EPA/AA ratio on reproduction, as well as egg and sperm quality [16, 19, 49].

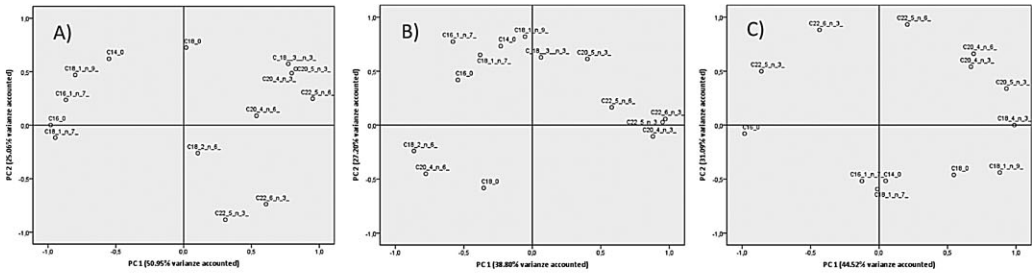
Despite the similarities found in the fatty acid profile of the ovaries from fish fed the ED and the ovaries from wild specimens, the TL content in ED fish was significantly lower than in wild fish and even than in nsCD fish. The reason for this difference between the two cultured groups is not clear. Taking into account that ED fish had been fed with commercial diet for its whole life (6 years) before the experiment, the drastic changes in its diet fatty acid composition might had led to a metabolic readjustment that may affect the lipid transport to ovary tissue, thus fish might have needed a longer period of adaptation to the ED. On the other hand, after 7 months of being fed with either the nsCD or the ED, not only the TL content but also the IGS was lower in both cultured groups than in wild fish and, in fact, no spawning were obtained during the first breeding season studied. This absence of spawning might be related with the ovary TL content, although there are other factors related to captivity conditions that also may be adverting spawning in cultured fish.

4.3 Eggs

During the second breeding season, after 15 months with the same dietary regime, fish fed with ED released eggs spontaneously from April till September, but none spawning was obtained from fish fed nsCD, which may suggest a positive effect of the experimental diet on ovary maturation and spawning. Although the spontaneous spawning of greater amberjack broodstock born in captivity is an important achievement which had not been reported to date, released eggs were not fertilized.

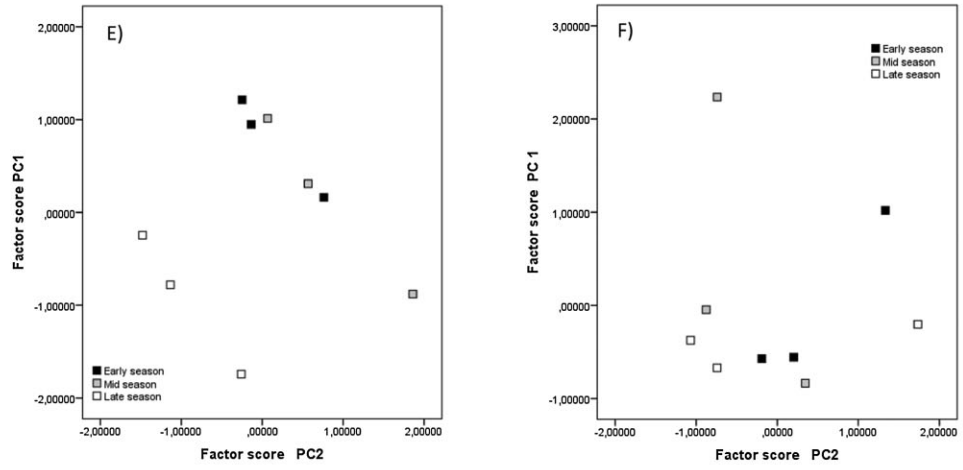
Given that males had reached maturity (releasing sperm under abdominal pressure) prior the first breeding season, the causes for the lack of fertilization in this study are not clear. Considering that this first work concerning nutrition and reproduction of *S. dumerili* broodstock born in captivity is only focused on females, important information is still lacking and much work remains to be done. The essential fatty acid content in broodstock diets and the ratios among them, affect the reproduction of both males and females [18], but both sexes may respond in a different manner [19]. As none studies have determined the lipid composition of wild males of this species, and their semen, and the lack of information regarding greater amberjack male reproductive behavior, further studies should be done to determine the possible causes associated with the reproductive failure observed in F1 males (lack of fertilization).

Regarding the lipid composition of the eggs obtained from fish fed on ED, they are neutral lipid rich eggs (>65% NL)



D)

Lipid Class	*Component*	*E- season*	*M-season*	*L-season*
Phosphatidylcholine	PC1	_	_	_
	PC2	_	_	_
Phosphatidylethanolamine	PC1	_	_	_
	PC2	_	_	_
Triacylglycerol	PC1	a	a	b
	PC2	_	_	_



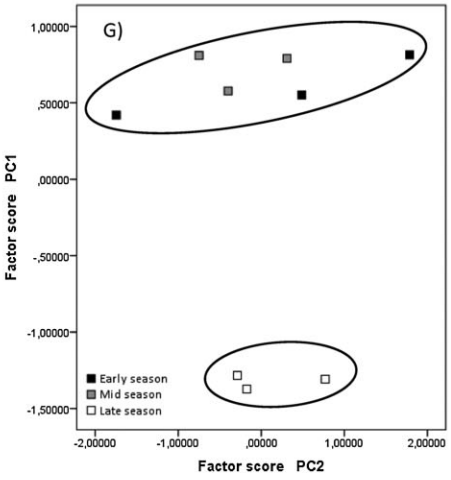


Figure 3. Principal component analysis (PCA) of fatty acids of phosphatidylcholine, phsphatidyethanolamine, and triacylglycerides fractions of non-fertilized eggs obtained from cultured specimens of *Seriola dumerili* fed on the experimental diet (ED) along the spawning season [(■) Early-season; (■) Mid-season; (□) Late season]. (A–C) Component loading plots (graphical representation of the correlation between each specific variable and the principal components PC1 and PC2). (A) PCholine, (B) PEthanolamine, (C) Triacylglycerides. (D) Results obtained by submitting factor scores to ANOVA test. Different superscript letter (a and b) show significant differences ($p \leq 0.05$) among groups for the factor scores. (E–G) Factor score plots (graphical representation of individual scores for each PC). (E) PCholine, (F) PEthanolamine, (G) Triacylglycerides. Bold line ellipse represents different clusters according to ANOVA results.

and its fatty acid profile is characterized by having 16:0 as the major saturate fatty acid followed by 18:0, oleic acid (18:1 *n*-9) as the main monoene, and DHA as the largest HUFA, keeping some similarities with many other marine species [22, 23]. Although the fatty acid composition of eggs is relatively less influenced by the broodstock diet than other fish tissues, many studies have shown that egg fatty acid composition can be influenced by broodstock diets [15, 22]. In this particular case, the high proportions of oleic and linoleic acid in ED eggs reflects the high level of those fatty acids in the broodstock diet. On the other hand, despite that AA levels in ED ovary tissue are similar to those of wild ovaries, AA levels were considerably lower in ED eggs than in ovary tissue. As observed by Furuita et al. [50] in *Anguilla japonica*, suggesting that this HUFA is more critical for oocyte maturation and ovulation than it may be for embryo and larvae. In early stages of development, AA have a clear positive effect enhancing egg and larval quality and handling resistance in some species [16, 51–54], but can also produce a detrimental effect if it is in excess [16, 50, 55]. Since every species have their own requirements on AA and *n*-3 HUFA, further studies should be done to determine *S. dumerili* embryo and early larvae fatty acid requirements.

Eggs fatty acid composition did not greatly change throughout the spawning season although experience some minor changes, being a marginal reduction of EPA in the late season the most striking variation. Although eggs composition tend to be conserved, slight variations in fatty acid profile along the spawning season has been also reported in other species such as common snook [56], gilthead seabream [57, 58], and turbot [59], with a decline in *n*-3 HUFA in the late season in most cases and a little decrease in EPA, as observed in this study. These differences observed in the TL fatty acid profile were mainly due to differences in TG, but EPA reductions were found not only in TG but also in PC and PE.

5 Conclusions

Overall, the use of the experimental diet have shown some positive results, since it resulted in a ovary fatty acid profile of cultured female that more resembles that of wild females, and enabled spontaneous spawning of *S. dumerili* females born in captivity. However the lack of fertilization and the high level of 18:2 *n*-6 in ovary tissue and eggs indicate that further improvements are needed in the broodstock diet formulation in order to enhance the reproductive performance of this species in captivity.

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