

Spawning Performance and Eggs and Larvae Quality in European Sea Bass (*Dicentrarchus labrax* L.) Broodstock Fed with Krill or Pufa Enriched Diets

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Abstract: Dietary factors, such as the origin and quality of the protein used, or the fatty acid composition of broodstock diets has been studied during last years in order to produce good quality fish eggs and larvae. Previous studies in the European sea bass (*Dicentrarchus labrax*) have shown the effect of the Polyunsaturated Fatty Acids (PUFA) dietary composition on the reproductive performance. In another hand, krill, used as an alternative source of proteins, has improved the eggs and larvae quality in the red seabream (*Pagrus major*). The present study consisted in the evaluation of the spawning performance and the eggs and larvae quality throughout the two first reproductive cycles of captive European sea bass. Fish were fed with a control diet (ST), or similar diets including krill flour (K) or PUFA-enriched oil (RO). K diet induced, in comparison with ST, a fecundity improvement, at least during the first spawning period after the puberty. However, this krill positive effect on the fecundity improvement was not evident during the second spawning period. On his hand, the RO diet induced the best quality eggs and larvae, but reduced spawning parameters.

Key words: European sea bass (*Dicentrarchus labrax*), egg, larva, quality, fatty acids

INTRODUCTION

During the last years, an important number of studies has been developed on the relations between nutrition and reproduction in several fish farmed species, and one special effort has been paid on the production of good quality eggs and larvae required for the development of fish farming^[1,2].

Although several studies has been developed considering the origin and quality of the protein used in the fish diets, a big variation between the different results has been obtained, making difficult to establish conclusions on the different studied species^[8-12], including the European sea bass (*Dicentrarchus labrax* L.)^[5,6]. At least in part, this variation on the results could be caused by the fatty acid composition of the source of proteins. In fact, numerous studies have been developed on the effect

of the dietary polyunsaturated fatty acids (PUFA) composition on the fish reproductive performance^[7-10]. In the European sea bass, several studies have shown that n-3 series enriched diets have profound effects on female reproduction, influencing patterns of gonadal development, plasma levels of lipids and sex steroids, egg quality and lipid levels, fecundity, hatching and survival rates^[5,6,11-14]. However, only a few studies^[28-33] describe the influence of series n-6 PUFAs on the reproduction of teleosts.

The effect of the use of krill as a raw material for the fish feeding has been considered in just a few studies on the red seabream (*Pagrus major*)^[15,16]. These studies showed that fish fed with krill before or during the spawning season produced eggs and larvae with a better quality than fish fed trash fish. The antioxidant capacities of carotenoids as the astaxanthin, present on the krill

Table 1: Composition of experimental diets (% dry weight)

| | ST | K | RO |
|---------------|------|------|------|
| Proteins | 50.8 | 50.5 | 51.4 |
| Lipids | 20.9 | 23.0 | 20.3 |
| Carbohydrates | 14.3 | 12.6 | 15.0 |
| Ash | 10.0 | 9.5 | 10.2 |
| Fiber | 1.1 | 2.3 | 1.2 |

composition, has been used to explain these positive effects^[2]. However, just some studies have been carried out controlling the level of dietary carotenoid supplied in broodstock diets Izquierdo^[2]) and no information is available in this regard for the European sea bass.

The experimental design of the present study was planned to test the effects of n-6 PUFA and krill flour. The present study consisted in the evaluation of the spawning performance and the eggs and larvae quality throughout the two first reproductive cycles of captive European sea bass females.

MATERIALS AND METHODS

Animals and experimental conditions: In July of the first year of experiment, one group of 1+ old European sea bass, having around 250 g, were maintained under natural photoperiod and temperature conditions (ranging from 11.5 °C in January to 28 °C in August) within the facilities of the Instituto de Acuicultura de Torre de la Sal (east coast of Spain, 40° N and 0° E). Fish were placed in 8,000 l tanks supplied with aerated flow-through (21 l min⁻¹) sea water (37.8‰ salinity; pH 8.1-8.3).

After one year of adaptation, in June of the second year, fish were sexed in basis of their weight, considering bigger fish as potential females (540.0±7.6 g vs 516.4±15.7 g in the study of males), to establish experimental groups. Fish were tagged with passive integrated transponders (P.I.T. tags) injected into the epaxial muscle, and divided into 9 groups (n=27; 9 potential females and 18 males). In November sex rate was checked by intraovarian canulation and abdominal massage. This resulted in a reduction from 9 potential to 5-6 real females tank⁻¹.

Every month, during 2 consecutive years, fish weight and length was determined to study the possible effect of the diets on the fish growth, and to adjust fish daily intake.

Fish feeding: Fish were hand fed 5 days a week, once a day and with a daily intake between 0.3% (winter) and 2.2% (summer) body weight. Until the beginning of the experiment fish were fed with a commercial pelleted diet

Table 2: Fatty acid composition of experimental diets (as % of total fatty acids)

| | ST diet | K diet | RO diet |
|------------------------|------------|-----------|-----------|
| Fatty acid | | | |
| Saturated | | | |
| 14:0 | 5.8±0.1 | 6.4±0.0 | 3.8±0.1 |
| 16:0 | 12.2±0.1 | 13.4±0.1 | 18.7±0.2 |
| 18:0 | 1.6±0.0 | 1.55±0.0 | 4.5 ±0.0 |
| Total | 20.2±0.1 | 21.9±0.1 | 28.3±0.3* |
| Monounsaturated | | | |
| 16:1n-7 | 4.1±0.0 | 4.3±0.0 | 4.6±0.0 |
| 18:1n-7 | 1.5±0.0 | 2.3±0.0 | 2.2±0.0 |
| 18:1n-9 | 11.2±0.1 | 11.1±0.1 | 12.6±0.0 |
| 20:1n-9 | 13.4±0.0 | 11.8±0.1 | 3.8±0.0 |
| 22:1n-11 | 19.3±0.0 | 16.4±0.2 | 5.0 ±0.0 |
| Total | 51.0±0.1** | 47.9±0.1* | 30.0±0.1 |
| PUFA n-3 | | | |
| 18:3n-3 | 1.4±0.0 | 1.3±0.0 | 1.2±0.0 |
| 18:4n-3 | 2.4±0.0 | 2.4±0.0 | 1.2±0.0 |
| 20:5n-3 (EPA) | 5.8±0.0* | 7.4±0.0** | 5.6±0.0 |
| 22:6n-3 (DHA) | 7.8±0.0 | 7.9±0.0 | 19.5±0.1* |
| Total | 19.3±0.1 | 21.0±0.0 | 29.3±0.1* |
| PUFA n-6 | | | |
| 18:2n-6(linoleic) | 4.6±0.0 | 4.3±0.0 | 4.9±0.1 |
| 20:4n-6 (AA) | 0.4±0.0 | 0.5±0.0 | 1.4±0.1* |
| 22:5n-6 | 0.1±0.0 | 0.1±0.0 | 1.2±0.0* |
| Total | 5.5±0.0 | 5.0±0.1 | 7.9±0.1* |
| Total PUFAs | 24.8±0.1 | 26.4±0.0 | 37.2±0.3* |
| n-3:n-6 PUFA | 3.6 | 4.2 | 3.7 |
| AA:EPA | 0.06 | 0.10 | 0.25* |
| DHA:EPA | 1.3 | 1.1 | 3.5* |

Results are expressed as mean ± SEM of 3 replicate analyses of different pools of each diet. Only those fatty acids present at levels of more than 1% in at least one of the diets have been included. * Denotes significant difference between diets.

(EWOS, 7 mm pellet) with the following proximate composition: 45% proteins, 12% lipids, 3,5% cellulose, vitamins A,D,E and copper. Later, during 2 years (6/95-6/97) and so including two first consecutive reproductive periods, fish were fed with three different diets. The ST group received a commercial pelleted diet enriched with Northern hemisphere fish oil (EWOS Technology Centre, Livingston, UK). This diet emulated the most successful diet, from previous work, in terms of female reproductive performance^[14]. The RO group received a similar diet with the lipid component replaced with tuna orbital oil (Ropufa 30, Roche Product, Heanor, UK) which is relatively low in EPA and contains significantly more AA than standard fish oil. On his hand, the diet for the group K consisted in the ST diet but with 45% of the protein fraction substituted by Atlantic krill proteins.

Analyses: Composition of experimental diets is shown in Table 1. The protein content was measured by a standard colorimetric method^[39] after digestion with NaOH. The ash content was estimated by calcination in an oven at 600°C for 15 h. Fibre was determined gravimetrically prior to extraction in a Tecator 1020 Hot Extractor. Carbohydrates were calculated by difference. The total lipids were

extracted with chloroform-methanol 2:1 (v/v)^[40] dried at 40°C under nitrogen and determined colorimetrically^[41]. Material for fatty acid analysis of diets was stored in at least 10 volumes of chloroform- methanol 2:1 (v/v) containing 0.01% (w/v) butylated hydroxytoluene (BHT) as an antioxidant. Lipids were extracted using the method of Folch *et al.*^[40]. Fatty acid methyl esters were obtained by acid-catalyzed transmethylation^[41] and purified by thin layer chromatography. Samples were analyzed following Bruce *et al.*^[28]. Fatty acid composition of the three experimental diets is presented in Table 2.

Spawning performance and eggs and larvae quality:

Natural spawnings, fertilized in the tanks, were collected during two consecutive spawning seasons. Eggs were moved from egg collectors to 2 l graduated cylinders to separate floating (viable) and sinking (unviable) eggs, and the viability of every spawn was calculated as the percentage of viable eggs. To calculate the number of eggs in every spawn, the formula established by Navas^[42] was applied, and other parameters (length of the spawning period, absolute and relative fertility, percentage of spawning females, number of spawns female⁻¹ and eggs spawn⁻¹) were determined as described by Zanuy *et al.*^[43].

In the first spawning season, 50 viable eggs were collected from every spawn. Number of lipid droplets, egg diameter, and lipid droplet diameter were measured in them. Moreover, aliquots of 100 viable eggs were collected to determine the wet and dry weights after freezing at -80°C and lyophilizing during 5 hours.

Eggs were incubated following the method described by Carrillo *et al.*^[4]. Hatching rate, length of larvae at hatching, and percentage of deformed larvae (showing abnormal development) at hatching were recorded.

In the second spawning season, 100 viable eggs samples were collected, recording just the egg diameter, the number of lipid droplets, and the wet and dry weights. Cell symmetry was evaluated during the first cell divisions. Hatching rate was recorded but incubations were carried out using 96 well microtitre plates incubated at 16°C as described by Oyen *et al.*^[44]. The study of larvae biometry and deformities was performed at 10 days of incubation.

Statistics: Results are expressed as mean ± Standard Error (SEM). A one way ANOVA was applied to data followed by a Student-Newman-Keuls test for multiple

comparisons. When normality or equal variance failed, a one way ANOVA on ranks was used followed by a Dunn's test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Fish growth: Figure 1A and 1B show, respectively, the changes on the mean body weight and body length of females in the three experimental groups. No significant differences between groups were observed throughout the experiment.

During October and November, coinciding with the period of vitellogenesis, a progressive increase in the growth rate was observed in all females as a consequence of the ovarian recrudescence and oocyte development. This was followed by a reduction in weight as a result of the spawning (January-March). This reduction was more important during the second spawning season, probably due to the better reproductive performance developed by the females in comparison with the first spawning season.

Variations on the fatty acid or protein composition of the European sea bass broodstock diets showed no effects on the females growth. Navas^[42] and Cerdá *et al.*^[15] found no significant differences on the growth rate in broodstock fed with different ratios, different content of lipids or proteins, or when diets enriched in n-3 fatty acids were used during different periods on the year. These results suggest that, once the minimal requirements for survival and growth has been covered, females destine to oocyte development the rest of available resources. Considering our own results, can be concluded that the composition of the ST diet contains these minimal requirements.

The weight increase observed on the different groups of females was similar to that observed by Cerdá *et al.*^[13] in European sea bass broodstock fed with a natural diet consisting in trash fish. Moreover, Cerdá *et al.*^[13,14] observed, independently of the diet received by the females, a clear decrease on the daily growth rate during the final phase of ovarian recrudescence. Also in captured or farmed salmonids, an important decrease of growth has been observed during the gonad development^[12,45], as a result of the hormonal changes occurred during the gametogenesis process. Spawning performance: Table 3 shows the parameters registered on the spawns produced during the two consecutive reproductive periods, on the three

Table 3: Length of the spawning period (days), percentage of spawning females, number of spawns female⁻¹, eggs spawn⁻¹ (x10³), absolute fecundity (eggs female⁻¹ x10³) and relative fecundity (eggs kg female⁻¹ x10³) during both consecutive spawning periods and in the three experimental groups. Most data are expressed as mean SEM. Different letters indicate significant differences (*P*<0.05).

| | | ST | K | RO |
|--|-----|----------------------|----------------------|---------------------|
| Spawning period (days) | 1st | 19/1-16/3/96 (57) | 15/1-20/3/96 (65) | 3/1-20/3/96 (77) |
| | 2nd | 24/1-20/3/97 (56) | 24/12/96 (79) | 11/1-6/3/97 (55) |
| Spawning females (%) | 1st | 23.3±5.1 | 72.2±14.7 | 23.3±14.5 |
| | 2nd | 90.0±4.2 | 78.1±19.3 | 31.5±20.0 |
| Spawns female ⁻¹ | 1st | 0.35±0.16 | 1.06±0.16 | 0.48±0.25 |
| | 2nd | 1.50±0.60 | 1.30±0.40 | 0.80±0.20 |
| Eggs female ⁻¹ (x10 ³) | 1st | 69±30 | 181±79 | 58±29 |
| | 2nd | 637±278 | 431±127 | 238±107 |
| Eggs kg female ⁻¹ (x10 ³) | 1st | 93±38 | 254±102 | 80±40 |
| | 2nd | 482±164 | 343±82 | 192±76 |
| Eggs spawn ⁻¹ (x10 ³) | 1st | 230±30 a | 180±10 ab | 122±7 b |
| | 2nd | 412±41 | 327±30 | 308±43 |

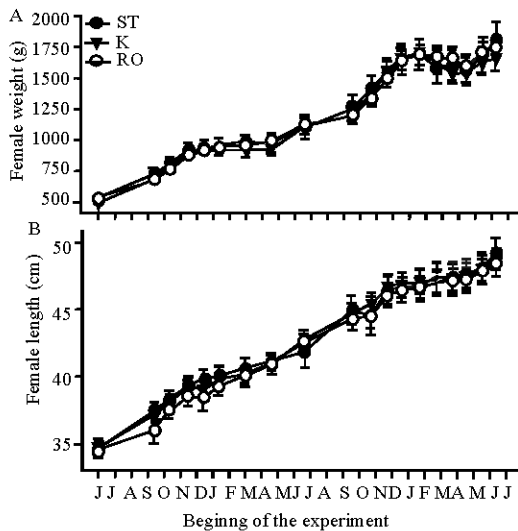


Fig. 1: Mean body weight (A) and body length (B) of females in the three experimental groups from the beginning of the experiment and during two consecutive spawning periods. Results are shown as mean±standard error (SEM)

experimental groups. K and RO (during first year) groups showed longer spawning periods than the group ST.

The percentage of spawning females during the first spawning period was near 23% in both ST and RO groups, while was three times higher (near 72%) in the group K although not significantly different. However, this situation was not repeated during the second spawning period, in which a slightly increase on the percentage of spawning females was observed in the K

and RO groups, while the ST group showed an important increase (from 23 to 90%).

Something similar happened with the number of spawns female⁻¹. During the first spawning period, the group K showed the highest number of spawns female⁻¹, followed by RO and ST. In the second spawning period, group K showed a slightly increase, while the RO group doubled and ST group multiplied by 4 the number of spawns female⁻¹ respect to the previous year.

In another hand, in both spawning periods the absolute fecundity (eggs female⁻¹ x10³) and the relative fecundity (eggs kg female⁻¹ x10³) were higher, although not significantly, in the groups ST and K in comparison with the RO one. In the first spawning period, the number of eggs spawning⁻¹ was significantly lower in the RO group in comparison with the ST group, while the K showed an intermediate value. This situation was similar during the second spawning season, but no significant differences were observed.

Anomalies on the results of spawning performance, and eggs and larvae quality, such as reduced number of spawnings, low fecundity, or low egg and larvae viability, were found during the first reproductive season. Nevertheless, during the second spawning season, one year after the puberty, all groups showed important increases of fecundity (mainly in the ST group) and the rates of egg viability and hatching. Previous studies^[3-46] found better reproductive performances in older females, probably due to better synchronization on the physiological factors involved in the process. This hypothesis is supported by the fact of finding a general improvement of the results during the second spawning season.

The increase in the number of eggs in the RO and K groups during the second spawning season was directly related with a clear reduction of the egg diameter. In the case of the European sea bass, previous studies^[13-42] as well as the present one, suggest that the increase of fecundity is concomitant with a reduction on the egg size, but without affecting significantly the egg and larvae viability. This fact has been corroborated in other fish species^[1]. However, in salmonids^[2], bigger and older females use to spawn bigger eggs.

The present study can not be statistically conclusive with respect to the possible relation between the egg size and the egg quality. In the European sea bass^[13] as well as in other fish species, bigger eggs produce bigger larvae^[47,48]. The importance of this fact is that bigger larvae can survive longer without food, they have a

higher range of captures, and they can avoid predators easier than little larvae^[1-49].

Quality of eggs and larvae: The percentage of viable eggs (with respect to the total amount of eggs in every spawn), as well as the egg diameter was higher, although not significantly, in the RO group during both reproductive cycles Table 4. All the groups showed around 10% of increase in the percentage of viable eggs during the second spawning period, one year after the puberty.

No differences were observed in the number of oil droplets egg⁻¹ or in the dry and wet weight of viable eggs. However, the percentage corresponding to dry weight over the total wet weight was significantly lower, at least during the first spawning period, in the eggs produced by females fed RO diet.

Although without significant differences, eggs of the RO group showed a higher hatching percentage, considering the viable fraction or the total spawn, during both consecutive spawning periods Table 5. This, together with the higher percentage of viable eggs Table 4, made that the hatching rate was approximately 20% higher in the RO group than in the other groups. However this effect did not caused a statistical difference. One increase in the hatching rate was observed in all the groups from the first to the second spawning period.

During the first spawning period, no differences between groups were found on the length of larvae after hatching (3 days) and after 8 days. However, in the second reproductive period, the group RO showed significant longer 10 days-larvae than the other groups.

No significant differences were found on the larvae deformity rate at 3 days (1st spawning period) or 10 days (2nd spawning period).

Previous studies with other fish species have found a direct relation between the egg viability and the blastomeres morphology in embryos at the 8 cells state^[50]. In the present study, this parameter was considered during the second spawning period and although no significant differences were found between groups, a parallelism between early cell symmetry and later eggs and larvae quality was found. Thus, cell symmetry, easily evaluable by fish farmers, could be considered as a usefull parameter in the early evaluation of spawn quality.

During the first spawning period, the group RO showed the better percentage of viable eggs and a high hatching rate, but its lower fecundity resulted in a lower reproductive efficiency. On his hand, the group K showed the highest fecundity, including the percentage of spawning females Table 3 and intermediate values of percentage of viable eggs and hatching rate Table 4 and 5, respectively, resulting in the best reproductive efficiency. Similar results has been obtained using krill as

a raw material for the red seabream (*Pagrus major*) broodstock feeding^[34,37,38]. These studies showed that fish fed with krill before or during the spawning season produced eggs and larvae with a better quality than fish fed trash fish. This positive effect has been attributed to the presence of phosphatidyl choline and astaxanthin from polar and nonpolar lipid fractions of raw krill, respectively^[35,36]. However, no positive effects has been observed in the case of the yellowtail (*Seriola quinqueradiata*), in which even a reduction in egg quality has been associated with high levels of astaxanthin^[51].

Results on the second spawning season were even better than showed for the K diet during the first spawning season. However, during the second spawning period, the K group showed intermediate values in the different parameters because the ST group showed the highest fecundity by increasing (until 90%) the percentage of spawning females and multiplying by 4 the number of spawns female⁻¹.

Table 4: Percentage of viable eggs, egg diameter (µm), number of oil droplets egg⁻¹, dry and wet weight of 100 eggs spawn⁻¹, and percentage of dry weight over wet weight, during both consecutive spawning periods and in the three experimental groups. Data are expressed as mean SEM. Different letters indicate significant differences (p<0.05).

| | | ST | K | RO |
|-----------------------------------|-----|------------|------------|-------------|
| Viable eggs (%) | 1st | 49.4±13.3 | 51.7±6.7 | 59.3±12.1 |
| | 2nd | 60.3±8.8 | 66.9±5.3 | 68.7±7.5 |
| Egg diameter (µm) | 1st | 1184±15 | 1196±9 | 1208±11 |
| | 2nd | 1184±12 | 1189±19 | 1201±26 |
| Oil droplets egg ⁻¹ | 1st | 1.7±0.3 | 1.4±0.2 | 1.8±0.2 |
| | 2nd | 1.5±0.1 | 1.5±0.1 | 1.4±0.1 |
| Dry weight egg ⁻¹ (µg) | 1st | 122±5 | 120±3 | 121±5 |
| | 2nd | 119±6 | 134±14 | 123±2 |
| Wet weight egg ⁻¹ (µg) | 1st | 936±25 | 981±26 | 1025±53 |
| | 2nd | 930±8 | 985±46 | 972±18 |
| Dry/wet weight (%) | 1st | 13.1±0.13a | 12.3±0.29a | 11.9±1.15 b |
| | 2nd | 13.0±0.52 | 12.4±0.15 | 12.7±0.17 |

Table 5: Percentage of hatching eggs, considering viable fraction and total spawn, during both consecutive spawning periods. Length of larvae at 3 days (1st spawning period), and at 10 days (2nd spawning period). Larvae deformity rate at 3 days (1st spawning period) and at 10 days (2nd spawning period). Data are expressed as mean SEM. Different letters indicate significant differences (p<0.05) between the three experimental groups.

| | | ST | K | RO |
|---------------------------------|-----|-----------|----------|-----------|
| Hatching rate 1 (viables) (%) | st | 48.0±19.2 | 45.7±9.5 | 65.8±13.9 |
| | 2nd | 56.4±7.7 | 55.9±6.9 | 76.0±3.9 |
| Hatching rate (total spawn) (%) | 1st | 22.7±14.5 | 30.8±5.6 | 46.0±10.7 |
| | 2nd | 33.0±7.5 | 36.1±5.5 | 53.8±7.0 |
| 3 days larvae length (mm) | 1st | 3.8±0.1 | 3.7±0.0 | 3.7±0.0 |
| | 2nd | 5.2±0.0b | 5.2±0.0b | 5.3±0.0a |
| Deformity rate at 3 days (%) | 1st | 50.7±19.2 | 38.1±5.5 | 40.5±4.4 |
| | 2nd | 16.5±3.3 | 18.6±2.2 | 11.8±2.5 |
| Deformity rate at 10 days (%) | 2nd | 79.1±5.2 | 81.2±5.7 | 92.0±2.0 |
| Cell Symmetry (%) | 2nd | 79.1±5.2 | 81.2±5.7 | 92.0±2.0 |

During this second period, the RO group showed again the better percentage of viable eggs and the highest hatching rate. Unfortunately, its percentage of spawning females was the lowest of the three experimental groups.

In fact, the best egg-larvae quality (egg size, high hatching rate and cell symmetry, low deformity rate, larvae length), as well as the low percentage of spawning females and the low egg production observed in the RO group can be related with the fatty acid composition of this diet. This diet was designed considering the conclusions on previous studies^[25,26,29,52-54] in which the importance of the ratios DHA:EPA and AA:EPA had been confirmed. The use in the RO diet of higher concentrations of AA and DHA, together with a lower concentration of EPA, signified higher relations AA:EPA and DHA:EPA, nearer to the necessities observed by the previous studies with European sea bass broodstock.

The role of DHA on the fish larvae development has been extensively documented. That could explain in part the good effect of RO diet on egg-larval quality. But most of the n-3 PUFA in the RO diet was obtained by the increase of DHA with respect to the ST diet, while EPA was reduced, resulting in a DHA:EPA ratio of 3.5. This unbalanced DHA:EPA ratio probably have had negative effects during oogenesis and this could explain the low percentage of spawning females and the low egg production.

In the other hand, AA has been determined a clear essential fatty acid for juvenile growth and survival^[55]. Koven *et al.*^[56] suggested the importance of dietary AA for improving resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. While Bruce *et al.*^[14] suggested that AA and its derived metabolites are probably involved in embryogenesis development of the immune system, hatching and early larval performance. However, RO diet showed reduced spawning parameters. Furuita *et al.*^[57] showed an improvement of Japanese flounder (*Paralichthys olivaceus*) egg-larvae quality and reproductive performance increasing the dietary AA levels (from 0.6 to 3.6% of total fatty acids), but overdose of AA (7.3%) caused significant negative effect both in egg production (eggs produced per spawning day per kg of female) and in egg quality and larval survival. Moreover, high n-6:n-3 diets have negative effects in salmonids such as marked cardiac myopathy, increased incidence of atherosclerotic lesions, decreased resistance to infection an altered ability of the liver to detoxify xenobiotics^[58].

In the present study, AA, n-3 and n-6 PUFAs were significantly higher in the RO diet. One of these high levels, or a combination of several of them, could be affecting negatively the reproductive capacity in the European sea bass. Previous studies in this species have probed the involvement of both n-3 and n-6 PUFAs in the control of oocyte maturation^[29,30], their participation in male reproductive performance^[44] and testis steroidogenesis^[45], as well as their regulatory role on the synthesis of prostaglandins (PGs)^[46]. In fish, several studies have demonstrated the role of PGs on gonadal steroidogenesis and ovulation^[29,30,59]. PGE₂ is derived from AA via cyclooxygenase and therefore the availability of AA is the limiting factor for PGE₂ and also PGF_{2α} production. This has been extensively demonstrated in the ovary of teleosts^[60,61]. On his hand, EPA competes with AA as a substrate for cyclooxygenase which results in a reduction in PGE₂ and PGF_{2α} levels and an increase in synthesis of PGE₃^[62]. This could be a complementary way to explain, at least in part, the good effect of RO diet on egg-larval quality.

Although the reproductive performance was better during the second reproductive cycle in all the experimental groups, the mean number of spawns female⁻¹ was just 1.5 in the best case (ST group), while this number can reach to 4, as has been observed in females fed with a natural diet consisting in trash fish^[63]. This result can be explained, at least in part, by the fact that not all the females reached to spawn, especially in the case of the RO group, which presented just 31.5% during the second spawning period. Moreover, the reduced final number of real females, a common problem in the captive European sea bass stocks, limited the statistically demonstrated conclusions of the present study, reducing to simple statistical tendencies some of the expected results. Despite this, results from the present study suggests that, in comparison with a standard diet, a krill-enriched diet can induce a fecundity improvement, at least during the first spawning period after the European sea bass puberty. On his hand, the use of high dietary concentrations of AA and DHA, together with a lower concentration of EPA, seems to improve the egg and larvae quality, but reduced spawning parameters in this species. New studies are required combining the effect of krill and PUFA on the reproductive performance of the European sea bass by designing a diet including both factors or by feeding krill-enriched diets until the puberty and changing to PUFA-enriched diets during the later spawning periods.

Moreover, the optimum AA:DHA:EPA ratio to formulate broodstock diets for this species remains unclear.

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