Fatty Acid Composition of Eggs and its Relationships to Egg and Larval Viability from Domesticated Common Sole (Solea solea) Breeders

Official name of the institution: Department of Veterinary Medical Sciences, University of Bologna.

Place where the work was carried out: Cesenatico (FC), Italy.

Title of Article: Fatty acid composition of eggs and its relationships to egg and larval viability from domesticated common sole (*Solea solea*) breeders.

Authors: Luca Parma, Alessio Bonaldo, Maurizio Pirini, Cinzia Viroli, Albamaria Parmeggiani, Erika Bonvini, Pier Paolo Gatta.

Abridged title: Egg quality in common sole.

Fatty acid composition of eggs and its relationships to egg and larval viability from domesticated common sole (*Solea solea*) breeders

L Parma[1]*, A Bonaldo[1], M Pirini[1], C Viroli[2], A Parmeggiani[1], E Bonvini[1], PP Gatta[1]

[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia, Italy; [2]**Department of Statistical Sciences "Paolo Fortunati"**, University of Bologna, Bologna, Italy

Content

The study of lipids and fatty acids (FAs) has been used in the assessment of egg quality because their composition can influence the fertilization rate, hatching, survival and growth of marine fish larvae. For these reasons, the lipid content (TL) and fatty acid composition of common sole (*Solea solea*) eggs were measured and correlated to egg and larval viability parameters throughout an entire reproductive season. Seventeen batches of fertile eggs obtained from natural spawning of captive breeders were characterized for the TL, FA profile, hatching rate (HR) and survival rate of larvae (SR) at 0–6 days post hatching (dph). The egg FA composition reflected the composition of the feed supplied to the broodstock during summer and autumn (before and during vitellogenesis) rather than that supplied during the spawning season. In general, the egg FA profile showed minimal differences **among the early, mid and late spawning periods (possibly due to the change of the diet and/or water temperature) indicating that it is possible to obtain a similar egg quality in terms of egg FA profile over two months of spawning**. Saturated FAs and monounsaturated FAs (MUFA), were positively correlated with HR, while TL, 22:6n–3 (DHA), 20:4n–6 (ARA), polyunsaturated FAs of the (n–3) series (n–3 PUFA), polyunsaturated FAs of the (n–6) series, were negatively correlated ($P \leq 0.05$). MUFA, 20:5n–3 (EPA), n–6/n–3 were positively correlated with SR, while DHA, n–3 PUFA, DHA/EPA, were negatively correlated ($P \leq 0.05$). In conclusion, the feed supplied before and during vitellogenesis has a major role in determining the egg FA profile in common sole. The relationships found between TL and FAs with egg and larval viability parameters differ from many other farmed marine fish species, which may suggest the need for a specific broodstock feed for this species.

Introduction

The ability to produce high-quality seed is a primary requirement for a successful aquaculture production (Migaud et al. 2013). In fact, the production of larval fish and their subsequent growth, development and health are highly dependent on the quality of egg available to the industry (Bromage and Roberts 1995). Egg quality can be defined as the egg's ability to be fertilized and subsequently survive and develop into a normal embryo (Bobe and Labbe 2010).

The chemical composition of fish eggs is often examined to evaluate their quality, as the eggs must satisfy the nutritional needs for embryonic and larval development (Furuita et al. 2002). In particular, the study of lipids and fatty acids (FAs) has been used in the assessment of egg quality because their composition can influence the fertilization rate, hatching, survival and growth of marine fish larvae (Tocher 2010). Polyunsaturated FAs (PUFAs) such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) have been shown to be essential for both reproductive control and embryo/larval development in many fish species (Izquierdo et al. 2001). They have also been found to be a source of stored metabolic energy, structural components during organogenesis and precursors of physiologically active molecules such as prostaglandins and other eicosanoids (Sargent 1995; Tocher 2003). Broodstock nutrition in marine fish species has been shown to affect egg nutrients qualitatively and quantitatively, and among those, lipids are the most studied because it is necessary to provide the correct FA amount to allow the production of robust and healthy larvae (Migaud et al. 2013). On the other hand, the determination of the optimal egg FA content, ensuring larvae of good quality, will give important indications for the ideal broodstock diet.

Recently, some studies have indicated the possibility of formulating correlations and interrelations between the FA composition of eggs and embryo/larval survival and that the qualitative and quantitative aspects of FAs can be used as potential indicators for predicting the quality of eggs in the teleost (Samaee et al. 2009, 2012).

Common sole (*Solea solea*) is a potential candidate marine fish species for European aquaculture, due to a high demand by consumers, high market value and in order to preserve its highly exploited wild stocks. To date, the aquaculture production of this species has been very scarce (Howell et al. 2011). The improvement in the control of reproduction and the standardization of the production processes to guarantee the availability of high-quality larvae and juveniles have been recognized as necessary for the development of its culture (Howell et al. 2011). Recent studies were carried out mainly focusing on the nutrition, feeding and physiology of larvae (Bonaldo et al. 2011; Ferraresso et al. 2013; Parma et al. 2013) and genetic selection of breeders (Blonk et al. 2010). Otherwise few studies have investigated the quality of eggs in domesticated breeders of this species and indications of the FA content of broodstock diets affecting egg quality are lacking.

To gain further knowledge about the effects of egg FA composition on egg and larval quality, the objectives of this study were: 1) to analyse and characterize the FA composition of common sole (*Solea solea*) eggs obtained from domesticated broodstock during an entire spawning season; (2) to assess changes in egg quality during the spawning season; (3) to define the relationship between FAs and egg viability parameters and larval survival; 4) to provide useful information for broodstock feed in common sole.

Materials and Methods

Broodstock management

The common sole broodstock used in this study (n = 24; sex ratio male:female = 1.2:1; male average weight: 387.4 ± 106.9 g; female average weight: 683.0 ± 173.1 g; **rearing density: 1.8 kg/m^3)** were captured at the juvenile stage in the Northern Adriatic Sea and were adapted to captivity for 5 years in an indoor elliptical tank of 7 m^3. The tank was supplied with natural seawater (36.0 ± 2.0 g L^{-1}) and connected to a closed recirculation

system. The water temperature (7.5–16.0 °C) was regulated according to the seasonal values recorded on the seabed of the Northern Adriatic (Arpa Emilia Romagna, Cesenatico, Italy). The photoperiod was modulated once a week according to the natural fluctuation at 44 3′ 0″ N, 12° 62′ 0″ W by an artificial white light bulb (Philips Softone 40 W; 20 lux at the water surface). During the pre-spawning season fish were fed *ad libitum* 3 to 5 times a week (at 16:00 hours) with semi-moist pellets, while during the spawning season fish were fed *ad libitum* with live polychaetes (*Nereis virens*) (Topsy Baits, Wilhelminadorp, The Netherlands) (2 to 4 times a week) and with fresh mussels *Mytilus galloprovincialis* (once a week). **The semi-moist pellets were formulated using the following ingredients: fish meal (450 g kg^{-1}); boiled and frozen mussels, *Mytilus spp.*, (450 g kg^{-1}); fish oil, Marinol D-40, Stepan Specialty Products B.V., Koog aan de Zaan, The Netherlands, (35 g kg^{-1}); water (36.5 g kg^{-1}); carboxymethylcellulose (11 g kg^{-1}); vitamin and mineral premix (17.5 g kg^{-1}). Vitamins were manufactured to supply the following vitamins (mg or IU kg^{-1} diet): vitamin C (500 mg), vitamin A (7500 IU), vitamin D3 (1125 IU), vitamin E (225 mg), vitamin B1 (12 mg), vitamin B2 (24 mg), vitamin B6 (24 mg), vitamin B12 (0.02 mg), vitamin K3 (7.5 mg), vitamin H (0.2 mg), vitamin PP (90 mg), folic acid (4.5 mg), pantothenic acid (72 mg), BHT (150 mg). Mineral mix was manufactured to supply the following elements (mg kg^{-1} diet): manganese oxide (22.5 mg), zinc sulphate monohydrate (135 mg), ferrous sulphate (60 mg), copper sulphate pentahydrate (7.5 mg), potassium iodide (3 mg), betain (600 mg). The ingredients were finely ground, mixed and pelleted using a laboratory pelleting machine (La Monferrina, P12, Asti, Italy) with 5 mm ring die.** The diet was produced every 2 months and stored at -32 °C until use. The proximate and FA composition of the semi-moist pellets, polychaetes and mussels are shown in Table 1.

Table 1

Spawning and sampling

During spring (March–May) the fish were induced to spawn naturally by increasing the water temperature (from 7.5 °C to 10.5–11.0 °C) over 3 consecutive days. The temperature was then maintained in the range of 10.0–11.2 °C for 10–15 days and thereafter it was reduced to 7.5–8.0 °C (1.5 °C day^{-1}) for 10 days to suspend the spawning. **This procedure, which involves water temperature fluctuations, was repeated for 3 times and it was necessary to stimulate the spawning of successive gamete batches along the reproductive season according to the standard protocol adopted in our laboratories and as previously reported for this species (Blonk et al. 2009). This procedure produced 3 spawning periods (early period, mid-period, late period) within the spawning season.** Spawns occurred at night and all the eggs spawned daily were collected in the morning (09:00) by an egg collector and kept as a separate batch. An egg sample (~ 300 eggs) from each batch was checked under a microscope to check for the presence of early cell division. Afterward, the total weight (TW) of each batch was recorded. Each batch was then separated into floating eggs, which were considered to be fertilized and of good quality (according to Anguis and Canãvate 2005; Lund at al. 2008), and sinking eggs using a 2 L calibrated cylinder at 10 °C and 38‰. The volumes (ML) of both fractions were recorded after complete separation (~ 15 min) and the floating rate (FR) was estimated. For lipid and FA analysis a representative subsample of 20 g of eggs from the floating fraction of each batch was collected, placed in a sieve, rinsed with distilled water and dried with blotting paper. The samples were weighed and stored at -80 °C until use. FR was calculated as a percentage of the floating egg volume compared to the total egg batch volume. The total egg number of each batch was estimated considering 550 egg $grams^{-1}$ (Blonk et al. 2009).

Determination of egg and larval viability parameters

Three representative egg subsamples from the floating fraction of each egg batch were randomly placed in three 96-well cell culture plates using a Pasteur pipette (one egg placed in each well filled with UV treated seawater, 38‰) and incubated in the dark at 11.0 ± 1.0 °C, using the method adopted by Samaee et al. 2009. The plates were inspected daily under a stereomicroscope and viable (floating with a translucent aspect) eggs and larvae were recorded to determine the hatching rate (HR) and survival rate (SR) until 6 days post hatching (dph). During the stereomicroscope observations (~ 10 min each plate) the plates were placed in a water bath at 10 °C to avoid water temperature fluctuations. HR was calculated as a percentage of the initial number of eggs incubated in each plate (n = 96), while SR at 1, 2, 3, 4, 5 and 6 dph was calculated as a percentage of the number of hatched larvae. All experimental procedures were evaluated and approved by the Ethical-Scientific Committee for Animal Experimentation of the University of Bologna, in accordance with the European Directive 2010/63/UE on the protection of animals used for scientific purposes.

Chemical analysis

The moisture content (AOAC 2000a) of the feedstuff (semi-moist pellets, fresh mussels and polychaetes) was determined in duplicate whereas the eggs were freeze-dried in a lyophilizer system, Heto Drywinner (Heto-Holten A/S Allerod, Denmark), and, after 24 h, were dried in an oven at 60 °C for 5 h. Crude protein (AOAC 2000b) and ash (AOAC 2000c) analyses were carried out in duplicate for the semi-moist diets, fresh mussels and polychaetes. Lipid extraction of eggs, semi-moist pellets, mussels and polychaetes was performed according to Bligh and Dyer (1959), using methanol and chloroform as solvents. FA methyl esters (FAMEs) from total lipid were prepared using methanolic sulphuric acid (1%) according to Christie (1989). Chromatographic analyses were carried

out with a Varian 3380 gas chromatograph (Varian Inc. Palo Alto, USA) equipped with a DB-23 J &W Scientific (30 m×0.25 mm) fused silica capillary column, a split injector at 230 °C and a flame ionization detector at 300 °C. The carrier gas was nitrogen at a flow rate of 1.2 ml min^{-1}. The oven temperature was set in a programmed mode from 150 °C to 230 °C at 5 °C min^{-1} and final isotherm. Data were processed using a Varian Star Chromatography Workstation. FAs were identified by comparing the retention times of unknown FAME with those of known FAME standard mixtures (Sigma-Aldrich Corp., St. Louis, MO, USA; PUFA No1, Marine Source, and PUFA No3, Menhaden Oil, SUPELCO, Inc., Bellefonte, PA, USA).

Statistical analysis

TL and the FA composition of the eggs spawned in different periods of the spawning season were analysed using one-way ANOVA followed by Tukey's *Multiple Comparison Test*. In order to evaluate the correlations and interrelations between the FA composition of the eggs and embryo/larval survival, linear regression analysis was performed using the software R version 3.1.0. More precisely, we separately tested the impact of the content of each FA component with respect to the % of the eggs hatched and % of the larvae surviving at 1, 2, 3, 4, 5 and 6 days post hatching, using a Student *t* statistical test within each regression analysis. Statistical significance was determined by considering the type I error at 5% for each analysis. In order to account for the natural and specific decay of the % of the larvae surviving at 1, 2, 3, 4, 5 and 6 days, described in Table 2, we incorporated in the regression model a polynomial function of order 4 so that the impact of the FA content on the percentage of the surviving larvae was purely evaluated without the confounding effect of the natural temporal trend.

Table 2

Results

Breeders spawned naturally following the increase in the water temperature and produced 17 batches of eggs at the cleavage stage (approx. 5–10 h post fertilization). Average data concerning the parameters of the egg batches produced in different spawning periods are shown in Table 2. The average TL content and the FA content and composition of the 17 egg batches analysed are shown in Table 3.

The TL and the FA composition of the eggs spawned during the early spawning season, mid-season and late season is shown in Table 3. The significant relations found between FAs and HR and SR at 1–4 dph are shown in Figs. 1 and 2. SFA, MUFA, were positively correlated with HR, while TL, ARA, DHA, n–6 PUFA, n–3 PUFA, were negatively correlated (Fig. 1 a, b). MUFA, EPA, n–6/n–3, were positively correlated with SR, while DHA, n–3 PUFA, DHA/EPA were negatively correlated (Fig. 2 a, b).

Table 3

Discussion

The reproductive performance in terms of the total egg production obtained in the present study was similar to that presented in a previous study of the same species (Lund et al. 2008) and the results of HR were slightly higher than those reported by Lund et al. (2008) and Blonk et al. (2009). The egg TL content recorded in the present study (18.78 ± 0.93% DW) was similar to that found in other cultured marine species such as: Senegal sole (*Solea senegalensis*), Japanese flounder (*Paralichthys olivaceus*), common dentex (*Dentex dentex*), gilthead sea bream (*Sparus aurata*) and white sea bream (*Diplodus sargus*) (Mourente and Vázquez 1996; Furuita et al. 2003; Cejas et al. 2004; Gimenez et al. 2006; Jerez et al. 2012), showing a lipid content of egg dry mass higher than 15%. The FA content was largely formed by n–3 PUFA (37.42%) followed by MUFA (23.50%), SFA (20.19%) and n–6 PUFA (9.66%). A similar FA profile was also reported by Lund

et al. (2008) for the same species except for the SFA (31.9–33.3 % TFA), which was higher than in the present study. A high level of n–3 PUFA was generally observed in the eggs of marine species (Tocher 2003), indicating the high requirement during the embryo and early larval stages (Tocher 2010) and reflecting the high levels of n–3 PUFA usually present in the formulated diets or in the natural feed for broodstock. DHA (26.8% total FAME), 16:0 (15.1%), 18:1 n–9 (12.3%), 18:2 n–6 (5.2%), were the most abundant egg FAs, which is in line with previous finding on this species (Lund et al. 2008).

Figure 1 a, b.

Figure 2 a, b.

A high proportion of DHA is consistent with general findings in marine fish larvae (Reitan et al. 1994; Nass and Lie 1998; Morais et al. 2004), and a high content has been related to specific requirements for DHA during the early larval development of neural tissues such as the brain and retina, as these tissues are highly enriched in DHA (Silversand et al. 1996). With regard to the DHA/EPA ratio, the values are in accordance with those found by Lund et al. (2008) in the eggs of wild broodstocks, and are consistent with selective catabolism of EPA relative to DHA in fatty acid oxidative processes producing energy for gonadogenesis (Sargent et al. 2002). Lund et al. (2008) discriminated wild from cultured common sole eggs based on FA composition and they found that the eggs from the cultured stock were higher in 18:2n–6, 18:3n–3 and 20:1n–9 content while the wild eggs were higher in 16:1n–7, ARA and EPA, due to dietary input. With regard to these specific FAs, our results showed an intermediate similarity to this classification. In fact, the levels of 18:2n–6, 18:3n–3 and 16:1n–7 in the present study were similar to those reported by Lund et al. (2008) for the cultured stock, while the levels of EPA, ARA and 20:1n–9 showed similarities to those reported for the wild. This might confirm that the feed composition supplied to the broodstock plays a determinant role in assessing the egg FA composition rather than the origin of the broodstock.

The differences in egg lipid content and FA composition observed between the early, mid- and late spawning season were minimal and mainly related to a decrease in TL, an increase in SFA (mainly 14:0 and 16:0) and a decreasing tendency of n–6 PUFA (mainly ARA and 18:2 n–6). The MUFA were stable over the spawning season **while n–3 PUFA and DHA, although not statistically significant, showed a decreasing trend at the end of the spawning season. To our knowledge, there are no study which quantified changes in the egg FA composition over the spawning season for this species**. Some of the differences found in the present work such as for SFA, n–6 PUFA and the DHA trend seem to be in relation to the shift of food from the semi-moist pellets (pre-spawning) to mussels and polychaetes (spawning). However, in general, the egg composition reflected the composition of the semi-moist diet more than the mussels and polychaetes. This was particularly relevant for the TL content, n–3 PUFA, n–6 PUFA and DHA and it might indicate that the feed supplied during summer and autumn (before and during vitellogenesis) could play a major role in determining the egg fatty acid profile in common sole in comparison to the feed supplied during the spawning season. A similar observation was also suggested for the composition of turbot ovaries, which were more readily affected by the diet during the early stages of gonadal development (Izquierdo et al. 2001). **Furthermore the differences observed in some egg FA with the advance of the spawning season such as SFA and DHA may be also related to the water temperature. Environmental factors such as temperature affect the lipid composition of fish tissues (Olsen et al. 1999) as observed in salmon and carp where a decrease in water temperature has been associated with an increase in DHA and PUFA content, respectively (Kayama et al. 1986; Olsen and Skjervold 1995). A decline of unsaturated FA and an increased of SFA was also observed on the common snook (*Centropomus undecimalis*) eggs as the spawning season progresses, suggesting a reduction in egg membrane fluidity (Evans et al. 1998; Yanes-Roca et**

al. 2009), while the decrease in DHA has been suggested as an adaptive mechanism to reduce membrane fluidity with the increase in temperature occurred during the embryonic development of the walleye, *Sander vitreus* (Mejri et al. 2014). In our study the early spawning period occurred after a month in which temperature was kept at 7.5 °C while during the mid and late spawning season several water temperature fluctuation up to 10-11.2 °C occurred. Thus, these fluctuations in temperature could have influenced the egg FA composition as the spawning season progressed.

With regard to the relationship between egg/larval quality parameters and egg composition, TL was negatively correlated with HR with better results achieved around 17% of egg TL content. This is not in agreement with the results found in brill (*Scophthalmus rhombus*) and halibut (*Hippoglossus hippoglossus*) where the total lipid content of eggs was higher in high-quality eggs (Evans et al. 1996; Hachero-Cruzado et al. 2011) but the total lipid content of the eggs showed no significant differences between high and low quality in common dentex (Gimenez et al. 2006) and Atlantic cod (*Gadus morhua*) (Salze et al. 2005). However, in the present study, the lipid content of the eggs seems to be higher than that found in the eggs of wild specimens (90 g kg^{-1} of TFA) of common sole (Lund et al. 2008). Dietary lipid level is known to influence the lipid level of the eggs as reported for gilthead sea bream (Harel et al. 1992), and the natural preys of sole (polychaetes, amphipods and small bivalves) have a lower lipid content in comparison with a formulated diet. Thus, at the embryonic stage an excess of lipid content in the eggs may not be optimal for embryo development and hatching in common sole.

The n–3 PUFA, n–6 PUFA, DHA and ARA contents were negatively correlated with hatching rate, while DHA, n–3 PUFA and DHA/EPA were inversely correlated with the survival rate of larvae. These results are in contrast with other studies on other species that showed that egg quality criteria, including hatching and early survival, were

positively correlated with increased levels of the n–3 HUFA and ARA in gilthead sea bream (*Sparus aurata*) (Harel et al. 1992; Rodríguez et al. 1998), Atlantic cod (Salze et al. 2005) and European sea bass (*Dicentrarchus labrax*) (Bruce et al. 1999). In contrast with most of those observations, a recent study indicates that an excessively high DHA level in eggs has a negative effect on egg and larval quality and reproduction performance in the flatfish tongue sole (*Cynoglossus semilaevis*) (Liang et al. 2014). The authors also suggested that both low and high levels of n–3 HUFA, n–6 HUFA and ARA in the broodstock diets (and consequently in their eggs) might have a negative effect on the larval quality of that species. The DHA and ARA requirements of common sole at the larval stage are still not clear; however, high ARA levels induce albinism (Lund et al. 2008), and the requirements of DHA seem lower in comparison to most of the commercial Mediterranean fish species so that there is no need to enhance the *Artemia* DHA concentration as long as there is a sufficient provision of EPA (Bonaldo et al. 2011). Interestingly, Morais et al. (2014) found that nutrient supplementation with DHA and vitamins in the broodstock feed of Senegal sole improved the DHA content of the eggs but did not enhance larval survival and the performance of larvae at 17 dph. The same authors also suggested that Senegalese sole larvae are capable of regulating DHA biosynthesis as early as at hatching to counterbalance the lower levels of DHA in their eggs. In contrast, SFA and MUFA were generally positively correlated with the HR and SR of larvae. Eggs from wild broodstocks of common sole reported higher levels of both SFA and MUFA (Lund et al. 2008) than in the present results. These FAs are generally used as an energy source during the early development from fertilized eggs to yolk sac larvae of most pelagic species with a role in the formation of embryo/larva tissue and in providing enough energy for larval swimming and prey capture (Samaee et al. 2009).

In conclusion our results indicate that common sole eggs obtained from captive broodstock fed a practical diet are characterized by a high lipid content with high levels

of n-3 PUFA, in particular DHA, which is highly retained in the egg compared to EPA. The egg FA composition reflected the composition of the semi-moist diet more than the mussels and polychaetes, indicating that the feed supplied during summer and autumn (before and during vitellogenesis) has a major role in determining the egg FA profile in common sole. **In general, the egg FA profile showed minimal differences among the early, mid and late spawning periods (possibly due to the change of the diet and/or water temperature) indicating that it is possible to obtain a similar egg quality in terms of egg FA profile over two months of spawning**. SFA and MUFA content showed a positive correlation with egg quality, while n-3 PUFA, DHA, DHA/EPA and ARA content was inversely correlated with egg and larval survival. This is typically in contrast to many farmed marine fish species and may suggest the need for a specific broodstock feed for this species.

Acknowledgement

We would like to thank Fulvio Mongile for his technical assistance and Proof-Reading-Services.com Ltd (Letchworth Garden City, UK) for English language editing. The study was financed by the Italian Ministry for Agricultural, Food and Forestry Policies (MIPAAF); project title: "Studio di fattibilità dell'intero ciclo produttivo della sogliola comune *Solea solea*: analisi e risoluzione dei punti critici e valutazione economica" (*Feasibility study on the entire production cycle of the common sole Solea solea: analysis and resolution of critical aspects and economic assessment*), invito DG Pemac Segreteria Prot. Uscita 0042305, 25/11/2001.

Conflict of interest

The authors of this article confirm that they do not have any conflicting financial or non-financial interests in its content.

Authors' contributions

LP, AB and PPG conceived and designed the project. LP and EB conducted the fish rearing and sampling. MP carried out chemical analyses. CV carried out statistical analyses. LP, AB, AP and PPG wrote the manuscript.

References

Anguis V, Canãvate JP, 2005: Spawning of captive Senegal sole (*Solea senegalensis*) under a naturally fluctuating temperature regime. Aquaculture **243**, 133–145.

AOAC Method 920.153, 2000a: Official Methods of Analysis of AOAC International, 17th edn. AOAC International, Arlington, VA, USA.

AOAC Method 950.46, 2000b: Official Methods of Analysis of AOAC International, 17th edn. AOAC International, Arlington, VA, USA.

AOAC Method 981.10, 2000c: Official Methods of Analysis of AOAC International, 17th edn. AOAC International, Arlington, VA, USA.

Bligh EG, Dyer WJ, 1959: A rapid method of total lipid extraction and purification. Can J Biochem Physiol **37**, 911–917.

Blonk RJW, Komen J, Kamstra A, Crooijmans RPMA, van Arendonk JAM, 2009: Levels of inbreeding in group mating captive broodstock populations of common sole, (*Solea solea*), inferred from parental relatedness and contribution. Aquaculture **289**, 26–31.

Blonk RJW, Komen H, Kamstra A, van Arendonk JAM, 2010: Estimating breeding values with molecular relatedness and reconstructed pedigrees in natural mating populations of common sole, *Solea solea*. Genetics **184**, 213–219.

Bobe J, Labbe C, 2010: Egg and sperm quality in fish. Gen Comp Endocrinol **165**, 535–548.

Bonaldo A, Parma L, Badiani A, Serratore P, Gatta PP, 2011: Very early weaning of common sole (*Solea solea* L.) larvae by means of different feeding regimes and three commercial microdiets: influences on performances, metamorphosis development and tank hygiene. Aquaculture **321**, 237-244.

Bromage NR, Roberts RJ, 1995: Broodstock Management and Egg and Larval Quality. Blackwell Scientific Publications, Oxford.

Bruce M, Oyen F, Bell G, Asturiano JF, Farndale B, Carrillo M, Zanuy S, Ramos J, Bromage N, 1999: Development of broodstock diets for the European sea bass (*Dicentrarchus labrax*) with special emphasis on the importance of n–3 and n–6 highly unsaturated fatty acid to reproductive performance. Aquaculture **177**, 85-97.

Cejas JR, Almansa E, Jerez S, Bolanos A, Felipe B, Lorenzo A, 2004: Changes in lipid class and fatty acid composition during development in white seabream (*Diplodus sargus*) eggs and larvae. Comp Biochem Physio B **139**, 209-216.

Christie WW, 1989: Gas Chromatography and Lipids: A Practical Guide. In: The Oily Press, Ayr, UK.

Evans RP, Parrish, CC, Brown JA, Davis PJ, 1996: Biochemical composition of eggs from repeat and first – time spawning captive Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture **139**, 139–149.

Evans RP, Parrish CC, Zhu P, Brown JA, Davis PJ, 1998: Changes in phospholipase A(2) activity and lipid content during early development of Atlantic halibut (*Hippoglossus hippoglossus*). Mar Biol **130** (3), 369–376.

Ferraresso S, Bonaldo A, Parma L, Cinotti S, Massi P, Bargelloni L, Gatta PP, 2013: Exploring the larval transcriptome of the common sole (*Solea solea* L.). BMC Genomics **14**, 315-337.

Furuita H, Tanaka H, Yamamoto T, Suzuki N, Takeuchi T, 2002: Effects of high levels of n-3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder *Paralichthys olivaceus*. Aquaculture **210**, 323–333.

Furuita H, Yamamoto T, Shima T, Suzuki N, Takeuchi T, 2003: Effect of arachidonic acid levels in broodstock diet on larval and egg quality of Japanese flounder *Paralichthys olivaceus*. Aquaculture **220**, 725–735.

Giménez G, Estévez A, Lahnsteiner F, Zecevic B, Bell JG, Henderson RJ, Piñera JA, Sánchez-Prado JA, 2006: Egg quality criteria in common dentex (*Dentex dentex*). Aquaculture **260,** 232–243.

Hachero Cruzado I, Herrera M, Quintana D, Rodiles A, Navas JI, Lorenzo A, Almansa E, 2011: Total lipid and fatty acid composition of brill eggs *Scophthalmus rhombus* L. relationship between lipid composition and egg quality. Aquac Res **42**, 1011–1025.

Harel M, Tandler A, Kissil GW, Applebaum S, 1992: The kinetics of nutrient incorporation into body tissues of gilthead seabream *S. aurata* females and the subsequent effects on egg composition and egg quality. Isr J Aquacult-Bamid **44**, 127.

Howell B, Pricket R, Cañavate P, Mañanós E, Dinis MT, Conceição L, Valente L, 2011: Report of the V Workshop on the Cultivation of Soles. CCMAR, University of Algarve, Faro, Portugal (5–7 April).

Izquierdo MS, Fernandez-Palacios H, Tacon AGJ, 2001: Effect of broodstock nutrition on reproductive performance of fish. Aquaculture **197**, 25–42.

Jerez S, Rodríguez C, Cejas JR, Martín MV, Bolaños A, Lorenzo A, 2012: Influence of age of female gilthead seabream (*Sparus aurata* L.) broodstock on spawning quality throughout the reproductive season. Aquaculture **350–353**, 54–62.

Kayama M, Hirata M, Hisai T, 1986: Effect of water temperature on the desaturation of fatty acids in carp. Bull Jpn Soc Sci Fish **52**, 853–857.

Liang MQ, Lu QK, Qian C, Zheng KK, Wang XX, 2014: Effects of dietary n-3 to n-6 fatty acid ratios on spawning performance and larval quality in tongue sole *Cynoglossus semilaevis*. Aquacult Nutr **20,** 79-89.

Lund I, Steenfeldt SJ, Suhr KI, Hansen BW, 2008: A comparison of fatty acid composition and quality aspects of eggs and larvae from cultured and wild broodstock of common sole (*Solea solea* L.). Aquacult Nutr **14**, 544–555.

Mejri S, Audet C, Vandenberg GW, Parrish CC, Tremblay R, 2014: Biochemical egg quality in a captive walleye (*Sander vitreus*) broodstock population relative to ovulation timing following hormonal treatment. Aquaculture **431**, 99-106.

Migaud H, Bell G, Cabrita E, McAndrew B, Davie A, Bobe J, Herràez MP, Carrillo M, 2013: Gamete quality and broodstock management in temperate fish. Rev Aquac **5**, S194-S223.

Morais S, Narciso L, Dores S, Pousão-Ferreira P, 2004: Lipid enrichment for Senegalese sole (*Solea senegalensis*) larvae: effect on larval growth, survival and fatty acid profile. Aquacult Int **12**, 281–298.

Morais S, Mendes AC, Castanheira MF, Coutinho J, Bandarra N, Dias J, Conceição LEC, Pousão-Ferreira P, 2014: Aquaculture, **432,** 374-382.

Mourente G, Vázquez R, 1996: Changes in the content of total lipid, lipid classes and their fatty acids of developing eggs and unfed larvae of the Senegal sole, *Solea senegalensis* Kaup. Fish Physiol Biochem **15**, 221-235.

Nass T, Lie O, 1998: A sensitive period during first feeding for the determination of pigmentation pattern in Atlantic halibut, *Hippoglossus hippoglossus* L., juveniles: the role of diet. Aquacult Res **29,** 925–934.

Olsen Y, Skjervold H, 1995: Variation in content of Ω3 fatty acids in farmed Atlantic salmon, with special emphasis on effects of non-dietary factors. Aquacult Int **3**, 22–35.

Olsen RE, Løvaas E, Lie Ø, 1999: The influence of temperature, dietary polyunsaturated fatty acids, α-tocopherol and spermine on fatty acid composition and indices of oxidative stress in juvenile Arctic char, *Salvelinus alpinus* (L.). Fish Physiol Biochem **20**, 13–29.

Parma L, Bonaldo A, Massi P, Yúfera M, Martínez-Rodríguez G, Gatta PP, 2013: Different early weaning protocols in common sole (*Solea solea* L.) larvae: Implications on the performances and molecular ontogeny of digestive enzyme precursors. Aquaculture **414-415**, 26-35.

Reitan KI, Rainuzzo JR, Olsen Y, 1994: Influence of lipid composition of live feed on growth, survival and pigmentation of turbot larvae. Aquacult Int **2**, 33–48.

Rodríguez C, Cejas JR, Martin MV, Badia P, Samper M, Lorenzo A, 1998: Influence of n–3 highly unsaturated fatty acid deficiency on the lipid composition of broodstock gilthead seabream (*Sparus aurata* L.) and on egg quality. Fish Physiol Biochem **18**, 177-187.

Salze G, Tocher DR, Roy WJ, Robertson DA, 2005: Egg quality determinants in cod (*Gadus morhua* L.): egg performance and lipids in eggs from farmed and wild broodstock. Aquacult Res **36**, 1488–1499.

Samaee SM, Lahnstainer F, Giménez G, Estévez A, Sarg B, Linder H, 2009: Quantitative composition of vitellogenin derived yolk proteins and their effects on viability of embryos and larvae of common dentex (*Dentex dentex*), a marine pelagophil teleost. J Exp Zool A **311**, 735-751.

Samaee SM, Estevez A, 2012: Embryo and Larva Development in *Dentex dentex*, a Marine Pelagophil Teleost: An Endeavor to find a series of new fatty acid interrelations J. Exp. Zool A **317**, 55–72.

Sargent JR, 1995: Origin and functions of egg lipids: nutritional implications. In: Broodstock Management and Eggs and Larval Quality (ed. by N.R. Bromage & R.J. Roberts), pp.353-372. Blackwell Science, London, UK.

Sargent JR, Tocher DR, Bell JG, 2002: The lipids. In: Fish Nutrition, 3rd edn, (ed. by J.E. Halver & R.W. Hardy), pp.181-257. Academic Press, San Diego,VA, USA.

Silversand C, Norberg B, Haux C, 1996: Fatty acid composition of ovulated eggs from wild and cultured turbot (*Schopthalmus maximus*) in relation to yolk and oil globule lipids. Mar Biol **125**, 269–278.

Tocher DR, 2003: Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci **11**, 107-184.

Tocher DR, 2010: Fatty acid requirements in ontogeny of marine and freshwater fish. Aquacult Res **41**, 717–732.

Yanes-Roca C, Rhody N, Nystrom M, Main KL, 2009: Effects of fatty acid composition and spawning season patterns on egg quality and larval survival in common snook (*Centropomus undecimalis*). Aquaculture **287**, 335–340.

Corresponding author's address

Luca Parma, Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia, Italy, PO Box, Via Doria 5A, 47042, Cesenatico, FC, Italy. E-mail: luca.parma@unibo.it

Table and figure captions

Table 1

Proximate analysis (% of dry weight) and fatty acid composition (% of total fatty acid methyl esters) of the different types of food supplied to the broodstock. Data are expressed as the mean ± SD. *N* = number of samples.

Table 2

Average egg batch parameters obtained from common sole broodstock during the spawning season. Early season: batches spawned on 08/03, 11/03, 12/03, 13/03, 14/03, 15/03, 16/03, 17/03, 18/03. Mid-season: batches spawned on 27/03, 28/03, 29/03, 31/03, 06/04, 07/04. Late season: batches spawned on 25/04 and 01/05; FR: floating rate, % of the floating eggs; HR: hatching rate, % of the eggs hatched; SR-1/6: survival rate of larvae, % of the larvae surviving at 1, 2, 3, 4, 5 and 6 days post hatching.

Table 3

Lipid (% of dry weight) and fatty acid composition (% of total fatty acid methyl esters) of egg batches spawned during three different periods: Early season: 8/03–18/03, *N* = 9; Mid-season: 27/03–07/04, *N* = 6; Late season: 25/04–1/05, *N* = 2.

Figure 1

Graphs showing the significant relations ($P \leq 0.05$) of hatching rate (HR, %) with: (a) egg lipid content (% of dry matter, DW), SFA (saturated fatty acids) and MUFA (monounsaturated fatty acids) content (% of total fatty acid methyl esters, FAME) (*N* = 17); (b) n–6 PUFA (polyunsaturated fatty acids), n–3 PUFA, ARA (arachidonic acid)

and DHA (docosahexaenoic acid) content (% of total fatty acid methyl esters, FAME) (N = 17) in common sole fertilized eggs.

Figure 2

Graphs showing the significant relations ($P \leq 0.05$) of survival rate (SR, %) at 1, 2, 3 and 4 days post hatching (dph) with: (a) MUFA (monounsaturated fatty acids), n–3 PUFA (polyunsaturated fatty acids), n–6/n–3 content (% of total fatty acid methyl esters, FAME) (N = 17); (b) EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), DHA/EPA content (% of total fatty acid methyl esters, FAME) (N = 17) in common sole fertilized eggs.

Table 1.

	Semi-moist diet (N = 3)	Mussels (N = 3)	Polychaetes (N = 3)
Protein	62.10 ± 1.32	46.38 ± 12.6	59.07 ± 4.39
Lipid	16.40 ± 0.34	12.67 ± 2.78	14.75 ± 2.03
Ash	15.89 ± 0.30	7.45 ± 1.49	12.29 ± 3.17
Fatty acids			
Total SFA	20.30± 0.19	21.70 ± 1.43	22.28 ± 1.33
Total MUFA	20.90 ± 0.12	24.77 ± 8.33	28.94 ± 2.12
Total n–6 PUFA	11.25 ± 0.03	2.83 ± 1.61	6.62 ± 0.27
Total n–3 PUFA	39.62 ± 0.29	29.92 ± 3.74	11.68 ± 2.31
20:4 n–6 (ARA)	1.87 ± 0.03	1.82 ± 1.62	0.74 ± 0.10
20:5 n–3 (EPA)	8.78 ± 0.14	17.68 ± 2.58	6.93 ± 1.83
22:6 n–3 (DHA)	26.05 ± 0.39	7.77 ± 2.09	1.39 ± 0.18

Each value is expressed as the mean ± SD. N = number of samples; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; ARA: arachidonic acid; PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Table 2.

Spawning period	Water temperature at spawning (°C)	Eggs per day (no. of eggs)	FR (%)	HR (%)	SR-1 (%)	SR-2 (%)	SR-3 (%)	SR-4 (%)	SR-5 (%)	SR-6 (%)
Early season	9.8 ± 1.3	59722 ± 18084	49.5 ± 16.1	31.7 ± 12.8	92.1 ± 7.9	70.7 ± 20.7	40.4 ± 20.4	11.2 ± 9.9	3.1 ± 6.4	1.6 ± 3.1
Mid-season	10.7 ± 0.5	47194 ± 15736	49.6 ± 10.9	38.7 ± 18.6	91.3 ± 7.8	73.6 ± 15.7	33.4 ± 25.5	7.4 ± 8.3	1.1 ± 1.1	0.0 ± 0.0
Late season	11.2 ± 0.1	40964 ± 30739	24.6 ± 14.5	72.6 ± 6.9	94.8 ± 5.2	77.6 ± 24.3	42.8 ± 43.2	9.9 ± 14.0	0.8 ± 1.1	0.3 ± 0.4

Table 3.

	Early season	Mid-season	Late season	Total egg batches *N*=17
Lipid	19.13 ± 0.68[b]	18.82 ± 0.91[b]	17.03 ± 1.40[a]	18.78 ± 0.93
Fatty acids				
14:0	1.13 ± 0.08[a]	1.26 ± 0.08[b]	1.43 ± 0.07[c]	1.21 ± 0.13
15:0	0.35 ± 0.02	0.36 ± 0.02	0.43 ± 0.03	0.36 ± 0.03
16:0	15.30 ± 0.27[ab]	14.35 ± 0.73[a]	16.62 ± 1.04[b]	15.12 ± 0.89
17:0	0.11 ± 0.01	0.14 ± 0.02	0.47 ± 0.04	0.16 ± 0.12
18:0	3.35 ± 0.18	3.20 ± 0.32	3.66 ± 0.03	3.33 ± 0.26
Total SFA	20.23 ± 0.32[b]	19.32 ± 0.37[a]	22.61 ± 1.21[c]	20.19 ± 1.10
16:1 n–7	4.46 ± 0.32[a]	5.30 ± 0.48[b]	6.15 ± 0.53[b]	4.95 ± 0.71
18:1 n–9	12.61 ± 0.42	11.93 ± 0.75	11.85 ± 0.22	12.28 ± 0.63
18:1 n–7	4.33 ± 0.29[a]	4.98 ± 0.38[b]	4.36 ± 0.13[ab]	4.56 ± 0.43
20:1 n–11	0.24 ± 0.08	0.36 ± 0.13	0.40 ± 0.04	0.30 ± 0.11
20:1 n–9	0.79 ± 0.26	0.85 ± 0.08	0.83 ± 0.05	0.82 ± 0.19
20:1 n–7	0.32 ± 0.04	0.39 ± 0.07	0.38 ± 0.01	0.35 ± 0.06
22:1 n–11	0.15 ± 0.03	0.12 ± 0.02	0.12 ± 0.01	0.14 ± 0.03
22:1 n–9	0.10 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.02
Total MUFA	23.01 ± 0.74	24.01 ± 0.98	24.17 ± 0.76	23.50 ± 0.95
18:2 n–6	5.40 ± 0.30[b]	5.23 ± 0.38[b]	4.19 ± 0.05[a]	5.19 ± 0.49
20:2 n–6	0.96 ± 0.21	1.29 ± 0.53	1.10 ± 0.18	1.09 ± 0.37
20:4 n–6 (ARA)	2.41 ± 0.07[b]	2.09 ± 0.22[a]	2.10 ± 0.03[a]	2.26 ± 0.21
22:4 n–6	0.40 ± 0.04	0.41 ± 0.08	0.23 ± 0.03	0.38 ± 0.08
22:5 n–6	0.82 ± 0.05	0.65 ± 0.06	0.55 ± 0.05	0.73 ± 0.12
Total n–6 PUFA	9.99 ± 0.19[b]	9.65 ± 0.37[b]	8.18 ± 0.17[a]	9.66 ± 0.63
18:3 n–3	0.68 ± 0.25	0.95 ± 0.11	0.70 ± 0.00	0.78 ± 0.23
18:4 n–3	0.26 ± 0.04	0.35 ± 0.07	0.35 ± 0.02	0.30 ± 0.07
20:4 n–3	0.48 ± 0.02	0.42 ± 0.03	0.36 ± 0.01	0.44 ± 0.05
20:5 n–3 (EPA)	4.06 ± 0.23	4.19 ± 0.53	3.72 ± 0.09	4.07 ± 0.37
22:5 n–3	4.93 ± 0.22[a]	4.99 ± 0.49[a]	5.93 ± 0.27[b]	5.07 ± 0.46
22:6 n–3 (DHA)	28.13 ± 1.74	25.41 ± 2.70	24.69 ± 0.90	26.77 ± 2.47
Total n–3 PUFA	38.53 ± 1.73	36.31 ± 2.47	35.76 ± 1.24	37.42 ± 2.24
n–6/n–3	0.26 ± 0.01	0.27 ± 0.02	0.23 ± 0.00	0.26 ± 0.02
DHA/EPA	6.96 ± 0.72	6.20 ± 1.34	6.64 ± 0.08	6.65 ± 0.97
EPA/ARA	1.69 ± 0.11	2.04 ± 0.43	1.77 ± 0.07	1.82 ± 0.31
Unknown	8.25	10.71	9.28	9.24

Each value is expressed as the mean ± SD. Columns with different superscript letters for a given value are significantly different ($P \leq 0.05$). *N* = number of samples; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; ARA: arachidonic acid; PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

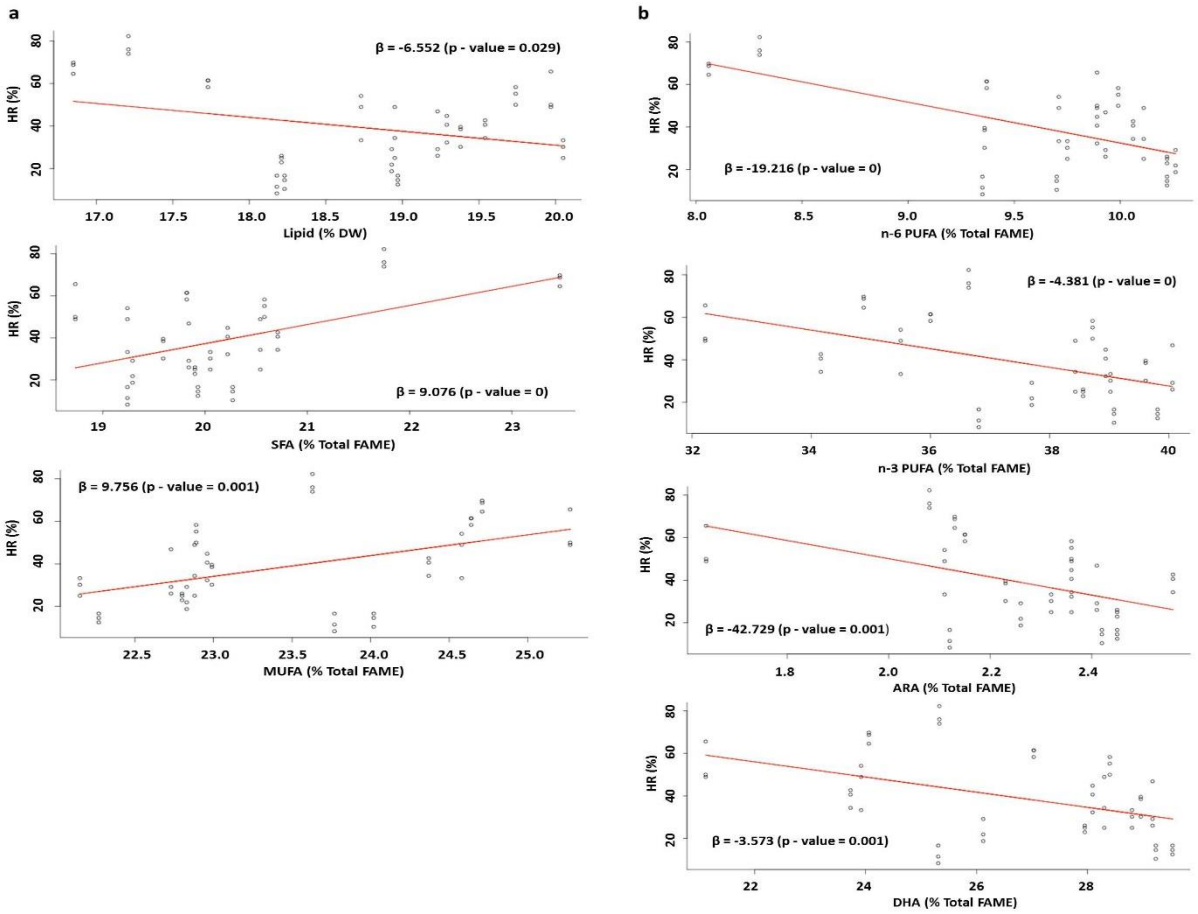
a
HR (%)
β = -6.552 (p - value = 0.029)
Lipid (% DW)
β = 9.076 (p - value = 0)
SFA (% Total FAME)
β = 9.756 (p - value = 0.001)
MUFA (% Total FAME)
b
β = -19.216 (p - value = 0)
n-6 PUFA (% Total FAME)
β = -4.381 (p - value = 0)
n-3 PUFA (% Total FAME)
β = -42.729 (p - value = 0.001)
ARA (% Total FAME)
β = -3.573 (p - value = 0.001)
DHA (% Total FAME)

Figure 1

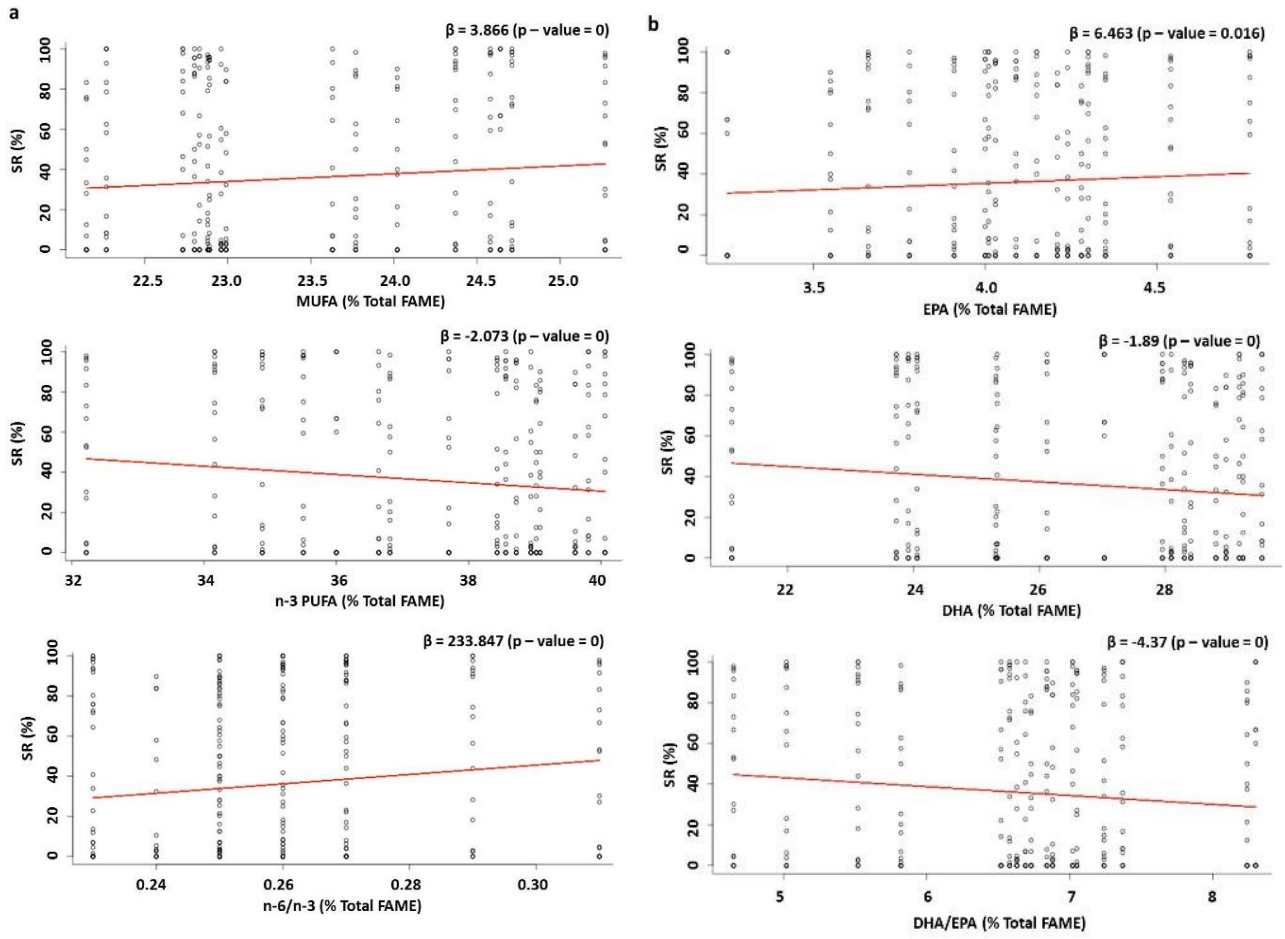
a
β = 3.866 (p – value = 0)
SR (%)
MUFA (% Total FAME)
β = -2.073 (p – value = 0)
n-3 PUFA (% Total FAME)
β = 233.847 (p – value = 0)
n-6/n-3 (% Total FAME)
b
β = 6.463 (p – value = 0.016)
EPA (% Total FAME)
β = -1.89 (p – value = 0)
DHA (% Total FAME)
β = -4.37 (p – value = 0)
DHA/EPA (% Total FAME)

Figure 2