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Relationships between spawn quality and biochemical composition of eggs and hatchlings of *Octopus vulgaris* under different parental diets

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ABSTRACT

Three frozen mono-diets (sardine, crab and squid) were supplied to *Octopus vulgaris* broodstocks, and their effects on the spawn quality and biochemical composition of eggs and hatchlings were studied. Squid and crab diets achieved superior spawn quality, producing in general significantly larger eggs, as compared to sardine diet. Similar differences were also observed in the biometric parameters of hatchlings (ventral and dorsal mantle lengths and dry weight) as well as in the survival of fasted hatchling which were greater in the squid and crab groups with respect to sardine treatment. On the other side, a strong and significant relationship between the mentioned biometrical measures and hatchlings survival was found, suggesting that eggs and hatchlings early biometrical measures can be utilized as a predictor for larval viability.
Statistical techniques as multivariate and correlation analyses were used to distinguish dietary groups based on protein content and lipid and fatty acid composition of achieved eggs and hatchlings as well as to relate this composition with spawn quality parameters. The analyses allowed to discriminate the nutrient composition of the early stages which was also found to be significantly correlated to the differences in the spawn quality of the different dietary groups, thereby underlining the importance of the protein content, and lipid classes like phosphatidylethanolamine, phosphatidylcholine, cholesterol and triacylglycerol, as well as docosahexaenoic (22:6n-3) and eicosapentaenoic (20:5n-3) fatty acids in the reproduction of *O. vulgaris*.

Statement of Relevance

It has been demonstrated that biochemical composition of the broodstock diet is a major determinant of successful reproduction and offspring survival in teleost fish (Watanabe & Vasallo-Agius, 2003; Bobe and Labbé, 2009). For *Octopus vulgaris*, our data suggest that the broodstock diet influenced the protein content and lipid composition of eggs and hatchlings, and these nutrients are related to the differences found in the spawn quality. A strong and significant relationship between the measured biometrical parameters and hatchlings survival was also found, suggesting that eggs and hatchlings early biometrical measures can be utilized as a predictor for larval viability.
On the other side, the utilization of nutrients during the embryo development can also be seen as an indicator of the nutritional requirements during the early stages of development and be useful for a future advance in the formulation of paralarvae artificial diets. Our results pointed to an embryo catabolism mainly based on proteins, as previously suggested (Lee, 1994; Villanueva and Norman, 2008). Data also suggest that triacylglycerol, sterol ester and phosphatidylcholine were specifically catabolized for energy and/or used to **de novo** synthesize lipid classes such as PS or PE. Within fatty acids, we found that arachidonic acid was strongly reduced (50–51%) during embryonic development. Further studies on the particular role of AA and the n–3 HUFA (mainly EPA and DHA) for early stages of *O. vulgaris* are needed, specially taking into account that these FAs are essential for this species (Monroig et al., 2012a,b).

1. Introduction

It is well recognized that the common octopus (*Octopus vulgaris*) is a potential species for aquaculture diversification because of its high

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market price and growth rate (3–15% body weight/day) in culture conditions, as well as short life cycle (12–18 months) and high fecundity (100,000–500,000 eggs/female) (Iglesias and Fuentes, 2014). Its great demand throughout different regions of the world is nowadays covered by the commercial fisheries since massive production of captive common octopus juveniles has not been achieved. The major bottlenecks are the high mortality observed in the paralarval stage (Iglesias et al., 2007, 2013), and the lack of an artificial diet that promotes acceptable growth and survival for this species or other cephalopods (Vaz-Pires et al., 2004; Quintana et al., 2008; Morillo-Velarde et al., 2012). Ongrowing and maintenance of juvenile and adult cephalopods are currently accomplished by feeding natural prey such as live, fresh or frozen fishes, crustaceans and/or molluscs.

A number of factors such as broodstock diet, genetics, maternal age and size and egg size among others, can influence the spawn quality (Izquierdo et al., 2001; Politis et al., 2014; Tocher, 2003). In particular, broodstock diet could be one of the most important since it provides the necessary nutrients to be utilized during the embryonic and paralarval development. In fact, it has been demonstrated that biochemical composition of the broodstock diet is a major determinant of successful reproduction and offspring survival in teleost fish (Almansa et al., 1999; Bobe and Labbé, 2010; Fernández-Palacios et al., 1997; Furuita et al., 2000, 2002; Izquierdo et al., 2001; Watanabe and Vasallo-Agius, 2003).

However, in spite of its importance for the management of offspring production, this topic has been scarcely researched in cephalopods. Food ration has been reported to significantly affect fecundity in *Idiosepius pygmaeus* (Lewis and Hodward, 1993) and *Enteroctopus megalocyathus* (Farías et al., 2011), but no differences in egg quality were found. On the other side, food ration had a significant effect on *Euprymna tasmanica* egg volume, those ones from high-ration females being larger (Steer et al., 2004). A second important aspect of broodstock diet is nutrient composition, since paralarvae need these nutrients for the lecithotrophic phase until the successful establishment of the exogenous feeding to support both homeostasis and development (Aby-ayad et al., 1997; Mourente and Vázquez, 1996; Ostrowski and Divakaran, 1991; Rainuzzo, 1993; Sargent et al., 1995). So, the egg nutrient composition can be a useful approximation to the nutritional requirements of paralarval stage as well (Navarro and Villanueva, 2000). In addition, the utilization of nutrients during the embryo development can also be seen as an indicator of the nutritional requirements during the early stages of development and be useful for a future advance in the formulation of paralarvae artificial diets. However, the determinant effect played by parental diet in egg quality is expected to intermingle with female-based effects when the analysis is extended to the individual level; this is why different authors dealing with egg quality introduce specific statistical techniques (Fukazawa et al., 2007; George, 1999; Giménez et al., 2006), i.e., nested ANOVA and multivariate analysis (Muñoz Serrano, 2003; Ruohonenen, 1998; Zar, 1999).

Protein and lipids play a major role in the physiology of cephalopods (Navarro and Villanueva, 2000, 2003; Navarro et al., 2014; Villanueva et al., 2004). In early stages of *O. vulgaris*, protein and lipid are used both as structural component and as substrate for energy metabolism (Quintana et al., 2006). Since protein is the main corporal component of the cephalopods and it has a vigorous amino acid metabolism (Lee, 1994; Villanueva and Norman, 2008) there is a large amino acid requirement for maintaining optimal growth and supplying energy. In addition, from their lipid composition and that of their natural prey, it can be deduced that early stages of cephalopod must require a food rich in polyunsaturated fatty acids (PUFA) (Monroig et al., 2012a,b; Navarro and Villanueva, 2000; Reis et al., 2014).

Since reproductive biology of cephalopods shows notable differences compared to teleost fish, the usual spawn quality criteria used in aquaculture facilities should be adapted to cephalopods. According to the general definition of spawn quality of Kjorsvik et al. (1990), as the potential to produce viable fry, we use quality parameters related to paralarval viability. In fact, survival of starved larvae has been widely used as an index of larvae viability, since this is a way of measuring the larval resistance against short periods without feed (Kamler, 2005). In consequence, the investigation of early biometrical measures of eggs and paralarvae as a proxy of larvae viability should be carried out. In a previous study, a significant correlation between egg biometry and paralarval biometry was found in *O. vulgaris* (Márquez et al., 2013); moreover, embryo and/or paralarvae survival data could advance a significant description of young stage quality (Migaud et al., 2013).

Specific studies of the broodstock nutritional requirements are necessary for cephalopod species (Farías et al., 2011; Lewis and Hodward, 1993; Steer et al., 2004). The study of egg and paralarval composition as influenced by the diet and its relation to the spawn quality has not been investigated in cephalopods; despite it could be one way to advance on broodstock dietary requirements.

The objectives of the present study were to determine the effects of three different diets (sardine, crab and squid) on spawn quality, biochemical composition of eggs and paralarvae, as well as on the utilization of nutrients during the embryonic development, focussing on lipid classes and fatty acids. In addition, the relationship between the spawn quality and biochemical composition of early stages was analyzed.

2. Material and methods

2.1. Experimental conditions and sample collection

O. vulgaris adults were collected in November 2003 in coastal waters near Huelva (SW Spain, Atlantic Ocean). Octopuses were captured by means of cephalopod pots and transported at the culture facilities of the Centro IFAPA "Agua del Pino" (Junta de Andalucia, Spain). Female ($n = 29$) and male ($n = 25$) mean wet weights were 789.0 ± 73.9 g and 801.9 ± 70.5 g, respectively. Adults were stocked in six breeding tanks (5000 L), at a density of nine individuals per tank (5 females and 4 males). A minimum of one pot per animal was placed into each tank. A flow-through open system of seawater (25 μm filtered) was established with a turnover rate of 144% of the tank volume per day. Water temperature ranged from 13 °C to 23 °C, salinity from 30 to 39 PSU, and photoperiod was natural and attenuated.

Broodstocks were fed with three frozen natural diets: crab (*Carcinus maenas*), sardine (*Sardina pilchardus*) or squid (*Illex* sp.). Since it is known that sardine as an exclusive diet promotes low growth on *O. vulgaris* (Cagneta and Sublimi, 1999), it was used like a negative control diet. Each diet was assigned to 2 breeding tanks. Broodstock disturbance was kept to a minimum to avoid any source of stress, so that breeders were not weighed. We started the experiment by supplying an initial ration of 360 g (per tank) for all the diets, approximately 5% of the wet biomass per tank. This ration was gradually modified according to the broodstock demand in order to assure that all broodstocks were fed ad libitum and, at the same time, minimizing the quantity of uneaten feed. All diets were supplied once per day in the morning. Food demands were recorded, ranging 360–1000 g/day for squid, 360–1200 for crab and 200–360 for sardine. The rearing plus spawning period lasted from November to July. Females of *O. vulgaris* attached egg strings inside the pot, taking care of them and avoiding food. When one spawn was detected, it was isolated in a separated tank and no more food was supplied during all embryonic development. Salinity and photoperiod in the isolation tanks were the same as those in the breeding tanks and average temperature was maintained at 20 ± 1 °C until hatchling succeeded.

Biometrical parameters on offspring were measured as has been described in Márquez et al., 2013. Four females for each treatment were selected and their spawns analyzed for egg biometry. From each spawn three strings were randomly selected. Eggs' wet weight was determined from one batch of eggs (around 30 eggs) from each string whereas 10 eggs per female were measured for length and width. All

eggs were sampled at days 10, 20 and 30 of incubation after the detection the spawn. In addition, three females per treatment were used for hatchling biometry. Newly hatched paralarvae (max. 24 hours-old) were gently extracted from the female's tank. 10 hatchlings per spawn were used for ventral and dorsal mantle length (MVL and MDL) and 5 batches of 40 hatchlings per spawn were used for dry weight (HDW) and organic content (HOC) measurements. Length measures were taken using a Nikon Labophot microscope (Nikon, Japan). For HDW, Whatman GF/C fiber-glass filters were used, washing samples with ammonium formate 0.5 M and drying at 100 °C for 24 h. The same batches were burnt at 450 °C during 4 h to obtain the HOC after 45 min in desiccators. All paralarvae were previously anesthetized using $MgCl_2$ 0.41 M solution.

Paralarval survival was compared among treatments using three females per dietary group. For each female, six batches of 200 newly hatched paralarvae (max. 24 h-old) were separated into 10 L methacrylate cylindrical tanks (15 cm diameter and 65 cm height). Three tanks were maintained under starvation and three tanks (control) were fed Artemia nauplii (EG) enriched with A1 DHA SELCO® (both Artemia cyst and SELCO were supplied by INVE; Dendermonde, Belgium). To reduce the effects of the stress, hatchlings were gently siphoned from the female's tanks and maintained in the 10 L tanks for 6 days (until 0% of survival was attained in the starvation group) at 20 ± 1 °C, 37 PSU, >6 mg O^2 L^{-1}, no water renovation and natural but attenuated photoperiod. On a daily basis, motionless paralarvae were gently siphoned out from the bottom of the tanks and counted as dead animals. The handling procedures were the same in all groups to avoid interferences in the survival rate. Survival rate was calculated at 3 days-old. Times for 75% and 100% mortality (T75 and T100) were also recorded.

Finally, samples were taken from the frozen natural diets supplied to the broodstock, octopus eggs of stages I–IV and hatchlings from each dietary group in order to analyze their biochemical composition.

2.2. Biochemical analysis

Biochemical analyses were carried out in the Departamento de Biología Animal and Departamento de Química Analítica, Nutrición y Bromatología of the La Laguna University (Tenerife, Spain). Moisture content, total protein (TP), total lipids (TL), lipid classes (LC) and fatty acids of total lipid (FA) were determined on the natural diets supplied as well as octopus eggs of stage I–IV and hatchlings samples. Moisture content was determined by using the method of Hortwitz. Kjeldahl method was used in order to obtain total nitrogen (AOAC, 1995) and protein content was calculated by using a conversion factor (Nx6.25). To obtain the total protein content, the non-protein nitrogen content was subtracted from the total nitrogen content. The determination of non-protein nitrogen (NNP) was analyzed according to the method recommended by the AOAC (1995), using 7.5% tricloroacetic acid to precipitate the protein. Lipid from the samples was extracted with chloroform:methanol (2:1 v/v) containing 0.01% of butylated hydroxytoluene (BHT) as antioxidant (Christie, 1982). The organic solvent was evaporated under a stream of gas nitrogen and the lipid content determined gravimetrically. Lipid classes were separated by one dimensional double development high performance thin layer chromatography (HPTLC) using methyl acetate/isopropanol/chloroform/methanol/0.25% (w/v) KCl (25:25:25:10:9 by vol.), as the polar solvent system, and hexane/diethyl ether/glacial acetic acid (80:20:2 by vol.) as the neutral solvent system. Lipid classes were quantified by charring with a copper acetate reagent followed by calibrated scanning densitometry using a Shimadzu CS-9001PC dual wavelength flying spot scanner (Olsen and Henderson, 1989). For the FA analysis, total lipid (TL) extracts were subjected to acid-catalyzed transmethylation during 16 h at 50 °C, using 1 mL of toluene and 2 mL of 1% sulphuric acid (v/v) in methanol. The resultant fatty acid methyl esters (FAME) were purified by thin layer chromatography (TLC), and visualized under UV light with 2′,7′-dichlorofluorescein in 98% (v/v) methanol, containing 0.01% BHT (Christie, 1982). FAME were separated and quantified by using a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (250 °C) and a fused silica capillary column Supelcowax™ 10 (30 m × 0.32 mm I.D.). Helium was used as carrier gas and samples were applied by on-column injection at an initial temperature of 50 °C. During each analysis, the oven was programmed to rise from 60 to 150 °C at a rate of 39 °C min^{-1}, and then to a final temperature of 215 ° C at 2.5 °C min^{-1}. Individual FAMEs were identified by reference to authentic standards and to a well-characterized fish oil (PUFA-3, Biosigma, Spain).

2.3. Statistical analysis

Results are presented as means ± standard deviations (SD). The data were checked for normal distribution with the one-sample Kolmogorov–Smirnov test (Zar, 1999) as well as for homogeneity of variances with the Levene's test (Zar, 1999). Percent data were arcsine transformed before analysis (Fowler et al., 1998) and log (1 + x) and root (1 + x) transformation were applied to the data when the previous test was not passed. Data obtained in the different groups were analyzed using a one way ANOVA followed by a posteriori post-hoc multi-comparison Tukey test (Zar, 1999). When a normal distribution and/or homogeneity of the variances were not achieved, data were subjected to a non-parametric Kruskal–Wallis test (Zar, 1999) and a Games–Howell post-hoc test was performed.

A nested analysis of variance (Muñoz Serrano, 2003) was applied to those parameters that could be individually measured (length and width of egg, as well as, LVM and LDM of the paralarvae). This analysis takes into account the three possible sources of variability (dietary treatments, replicating and sub-sampling) and was followed by a Tukey multiple comparison test. To analyze the effect of the broodstock diet on the paralarval survival under starved conditions, a comparison of the survival curves was performed by means of a chi-square test (Carrasco, 1995).

In addition, principal component analysis (PCA) and discriminant analysis of the LC and FA composition of eggs in stages I–IV and hatchlings were also used to explore the effects of dietary treatments. Possible relationships between FA profile of both octopus stages (egg and hatchling) and the spawning quality parameters were analyzed using the Pearson correlation. Specifically, the correlation analyses were carried out between the spawning quality parameters and the PCA axes achieved from LC and FA composition of eggs and hatchlings. After this, Bonferroni correction was applied to every test in order to assure the strength of the correlation obtained. In the particular case of TL and TP contents, Pearson correlation was directly used to look for possible significant correlations with the mentioned spawn quality parameters.

Table 1
Effect of the diet supplied to *Octopus vulgaris* broodstock on length, width and wet weight of obtained eggs at different days (10, 20 and 30) of development.

		Squid	Crab	Sardine
Length	Eggs 10d	2554 ± 68 a	2450 ± 82 b	2261 ± 149 c
	Eggs 20d	2508 ± 105 a	2388 ± 82 b	2281 ± 64 c
	Eggs 30d	2473 ± 60	2519 ± 82	2458 ± 286
Width	Eggs 10d	963 ± 70 a	926 ± 25 b	881 ± 34 c
	Eggs 20d	998 ± 103	958 ± 66	970 ± 64
	Eggs 30d	1010 ± 84	1143 ± 43 *	1169 ± 141 *
Wet weight	Eggs 10d	1.33 ± 0.01 a	1.33 ± 0.01 a	1.07 ± 0.06 b
	Eggs 20d	1.32 ± 0.14 a	1.34 ± 0.16 a	1.18 ± 0.05 b
	Eggs 30d	1.53 ± 0.44	1.71 ± 0.03 *	2.04 ± 0.57 *

Results of egg length (μm), width (μm) and wet weight (mg) are the average ± standard deviation (SD). Different letters in the same row mean significant differences ($P < 0.05$). For egg length and width, 10 eggs were measured per female. For egg wet weight, three batches of eggs were weighted per female (4 females/dietary treatment).
* Indicates significant differences compared to initial value at 10 days of development.

Table 2
Effect of the diet supplied to *Octopus vulgaris* broodstock on ventral (MVL) and dorsal mantle length (MDL), dry weight (HDW) and organic matter content (HOC) of obtained hatchlings.

	Squid	Crab	Sardine
MVL[1]	1519 ± 48 a	1534 ± 53 a	1458 ± 68 b
MDL[1]	2280 ± 59 b	2333 ± 54 a	2236 ± 85 c
HDW[2]	0.32 ± 0.01 a	0.29 ± 0.01 a	0.22 ± 0.04 b
HOC[3]	88.0 ± 3.9	88,3 ± 5.6	80.5 ± 12.3

Results are the average ± standard deviation (SD). Different letters in the same row mean significant differences ($P < 0.05$). For MVL and MDL measures, 10 hatchlings were measured per female. For HDW and HOC measures, five batches of 40 hatchlings were used per female (3 females/dietary treatment).
[1] Data in μm.
[2] Data in mg of dry weight.
[3] Data in percentage of dry weight (%).

To study the variation of biochemical composition during the embryonic development according to the diet supplied to the broodstocks, a t-Student test was applied within each dietary group.

Unless otherwise stated, statistical significance is taken to be indicated by P-values of less than 5%. The statistical analysis was carried out using the Statistix 8.1 for the nested analysis and SPSS 9.0 for the others.

3. Results

3.1. Effect of parental diet on offspring biometry and viability

Table 1 shows the results of length, width and wet weight through the embryonic development of the eggs obtained from broodstocks fed squid, crab and sardine. At day 10 of the development, the squid group showed the higher values in all parameters and the sardine group the lower values. Crab group showed values between squid and sardine for length and width and similar values to squid for wet weight. The mentioned differences diminished in the course of the embryonic development and no differences were observed at day 30 due to higher and significant increments of these parameters in sardine and crab groups with respect to squid group. In this sense, wet weight increased a significant 30% and 90% in crab and sardine groups respectively, whereas width increased 20% and 30% respectively but no significant increase was detected in the squid group. Finally, length showed the lowest increments with a significant 9% increment in sardine group.

The effect of the broodstock diet on new hatchlings was determined measuring the mantle length, both ventral (MVL) and dorsal (MDL), as well as dry weight and organic content (Table 2). In agreement with egg parameters at day 10 of development, hatchlings from sardine group showed significant lower values as compared to crab and squid except in organic content.

A hatchling survival over time curve under starvation was recorded for each dietary treatment (Fig. 1). The survival curve of the squid group

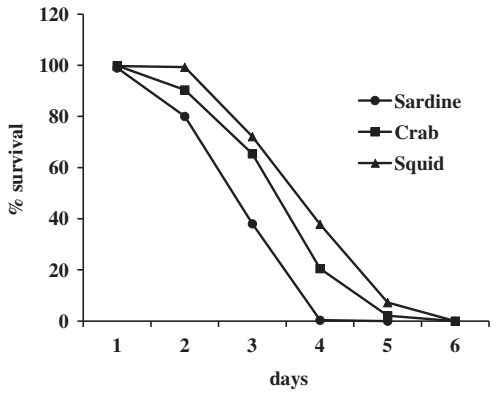

Fig. 1. Survival of starved hatchlings obtained from broodstock fed squid, crab or sardine.

Table 3
Spearman correlations between survival of starved hatchling and early biometrical variables measured on eggs (stages I–IV) and 0–24 hours-old hatchlings.

	EWW	HDW	HOC	L10	W10	MVL	MDL
Survival							
Spearman coef.	**0.778**	**0.824**	0.168	**0.766**	0.539	0.588	**0.723**
P-value	**0.023**	**0.006**	0.666	**0.027**	0.168	0.096	**0.028**

Bold values remark significant correlations ($P < 0.05$). The measured biometrical variables were: egg wet weight (EWW), hatchling dry weight (HDW), hatchling organic content (HOC), 10 days-old egg length (L10), 10 days-old egg width (W10), hatchling mantle ventral length (MVL) and hatchling mantle dorsal length (MDL).

was above that of the crab group, and the last one above that of the sardine group ($P < 0.001$). A correlation analysis clearly supported a strong and significant ($P < 0.05$) relationship between paralarval survival at the 4th post-hatching day (maximum life-period for sardine group of paralarvae) and biometrical parameters of initial eggs and hatchlings, such as egg length and wet weight and hatchling DML and dry weight (Table 3).

The results of the nested statistical analysis showed significant effects for the female factor regardless of the broodstock diet (with the exceptions of the length at day 20 and the width at days 10 and 20), although this variability was lower compared to that one due to diet (data not shown). On the other hand, there were no differences between subsamples in any parameter.

3.2. Effect of parental diet on offspring biochemical composition

Total protein content (TP) was significantly different between the three diets, being higher for squid and lower for crab (Table 4). A higher content for total lipid (TL) in the sardine was remarkable, although it was not statistically significant ($P > 0.05$) due to the high variability. In general terms, lipid class composition (% TL) of diets was characterized by a predominance of phosphatidylcholine (PC), cholesterol (CHO) and sterol esters (EE) in squid and crab and triacylglycerol (TG) in sardine (Table 4). Fatty acid (FA) composition of the three diets showed high levels of saturates (32–41%) and n-3 HUFA (26–32%) (Table 5). No relevant differences were observed except for n-6 FA series with a higher level in crab, particularly 20:4n-6 (AA).

Proximate and lipid class composition of eggs and hatchlings are presented in Table 6. Irrespective of the diet supplied to the broodstocks, a predominance of protein content was observed in eggs and hatchlings. Within the lipids, PC and CHO were the most abundant

Table 4
Total protein and total lipid contents (dry weight percentage) and lipid class composition (percentage of the total lipid) of the diets supplied to the *O. vulgaris* broodstocks.

	Diets		
	Squid	Crab	Sardine
Total protein	53.2 ± 2.3a	21.5 ± 4.7c	36.2 ± 5.1b
Total lipid	9.8 ± 0.6	6.7 ± 2.3	24.2 ± 17.8
Lysophosphatidylcholine	1.3 ± 0.6	1.6 ± 1.1	0.8 ± 0.9
Sphingomyelin	0.8 ± 0.5	0.6 ± 0.6	0.7 ± 0.5
Phosphatidylcholine	15.1 ± 3.2a	14.6 ± 4.0a	4.6 ± 3.3b
Phosphatidylserine	1.7 ± 0.4	1.0 ± 0.3	0.7 ± 0.7
Phosphatidylinositol	2.9 ± 0.9a	1.3 ± 0.7ab	0.6 ± 0.5b
Phosphatidylglycerol[1]	nd	2.8 ± 1.3	1.5 ± 1.8
Phosphatidylethanolamine	10.6 ± 1.8	8.2 ± 1.3	4.2 ± 4.8
Diacylglycerol	nd	3.2 ± 2.7	nd
Cholesterol	14.5 ± 1.4ab	16.5 ± 3.0a	7.9 ± 3.9b
Free fatty acids	29.8 ± 4.3a	16.7 ± 1.3b	15.9 ± 7.3b
Triacylglycerol	7.6 ± 3.4b	22.8 ± 6.2a	58.7 ± 27.3a
Sterol ester	15.5 ± 1.5a	10.6 ± 3.6ab	4.6 ± 5.9b
Total polar lipid	3.2 ± 0.6	1.9 ± 0.6	1.5 ± 0.8
Total neutral lipid	6.6 ± 0.8	4.7 ± 2.0	22.7 ± 18.4

Results represent means ($n = 4$) ± standard deviation (SD). Different letters in the same row mean significant differences ($P < 0.05$). nd, not detected.
[1] May also include phosphatidic acid and cardiolipin.

Table 5
Total fatty acid composition (% weight) of the diets supplied to the *O. vulgaris* broodstocks.

	Diets		
	Squid	Crab	Sardine
14:0	2.9 ± 1.7 b	1.4 ± 0.8b	7.0 ± 1.8a
15:0	0.6 ± 0.2	1.2 ± 0.6	0.8 ± 0.3
16:0	30.9 ± 5.0	20.9 ± 5.3	19.5 ± 7.2
16:1n-7	1.3 ± 0.8b	5.6 ± 3.6a	7.5 ± 1.8a
16:1	0.4 ± 0.3	1.5 ± 1.1	0.2 ± 0.2
18:0	5.2 ± 3.5	7.3 ± 0.7	3.7 ± 2.0
18:1n-9	7.2 ± 4.1	9.7 ± 1.6	10.4 ± 6.4
18:1n-7	2.7 ± 0.8	4.2 ± 1.3	2.6 ± 1.0
18:2n-6	1.3 ± 1.4	2.0 ± 1.4	1.7 ± 1.0
18:3n-3	0.5 ± 0.2	1.0 ± 0.9	1.1 ± 0.7
18:4n-3	0.7 ± 0.7	0.5 ± 0.3	3.2 ± 2.7
20:1n-9	4.9 ± 2.6	2.6 ± 0.9	4.4 ± 2.9
20:2n-6	0.3 ± 0.1ab	0.9 ± 0.5a	0.1 ± 0.1b
20:4n-6	0.6 ± 0.4	4.0 ± 2.9	0.8 ± 0.4
20:5n-3	10.1 ± 4.2	11.3 ± 4.1	11.0 ± 6.8
22:1n-9	0.9 ± 1.0ab	0.1 ± 0.2b	1.7 ± 1.0a
21:5n-3	0.5 ± 0.2ab	1.0 ± 0.7a	0.1 ± 0.2b
22:4n-6	0.2 ± 0.4ab	1.0 ± 0.5a	ndb
22:5n-6	0.2 ± 0.2	0.5 ± 0.1	0.2 ± 0.3
22:5n-3	0.5 ± 0.2b	1.9 ± 0.7a	1.6 ± 0.8a
22:6n-3	21.0 ± 5.3	12.0 ± 9.3	14.0 ± 7.2
UK	1.8 ± 0.7	2.3 ± 0.5	1.4 ± 1.0
Saturates	41.4 ± 7.5	33.2 ± 5.1	32.1 ± 7.8
Monoenes	18.8 ± 6.6	24.2 ± 6.7	28.1 ± 10.8
PUFA	42.9 ± 8.9	41.5 ± 7.4	41.6 ± 15.0
n-3	34.5 ± 10.4	30.0 ± 7.3	33.8 ± 18.1
n-6	3.1 ± 2.7b	8.8 ± 2.8a	3.2 ± 0.6b
n-9	13.5 ± 5.7	12.5 ± 2.0	17.4 ± 8.5
n-3 HUFA	32.2 ± 9.3	26.4 ± 8.3	27.6 ± 14.7
n-3/n-6	18.38 ± 11.80	3.82 ± 2.13	10.97 ± 6.53
EPA/DHA	0.46 ± 0.11	1.43 ± 1.08	0.77 ± 0.21
AA/EPA	0.05 ± 0.03	0.33 ± 0.22	0.08 ± 0.02
18:1/n-3H	0.27 ± 0.24	0.41 ± 0.21	0.85 ± 1.25

Results represent means (n = 4) ± standard deviation (SD). Different letters in the same row mean significant differences (P < 0.05). nd, non detected. UK: unknown. PUFA: polyunsaturated fatty acids. HUFA: highly unsaturated fatty acids. EPA: eicosapentaenoic acid (20:5n-3). AA: arachidonic acid (20:4n-6). DHA: docosahexaenoic acid (22:6n-3). 18:1/n-3H: ratio 18:1n-9/n-3 HUFA.

lipid classes. A general tendency was observed, not significant (P > 0.05), for lower content of protein, lipids and the main lipid classes in both stages of the sardine group in comparison to the other two groups. This tendency was not observed when data of lipid classes were represented as %TL (data not shown), but hatchling TG levels in squid and crab groups (6–8%) doubled that in the sardine (3%) one.

A significant decreased of TP (42–50%) was observed during the embryonic development for all the dietary treatments (data not shown). On the contrary, regarding to the lipid variations (11–29%), only the neutral lipids (NL) decreased significantly in the crab and sardine groups (34 and 40% respectively). As regards to particular lipid classes, also significant decreases of PC, TG and EE were observed for crab and sardine groups, being the relatively highest variations a 43% for PC and 78–79% for TG and EE in the sardine group. On the contrary, CHO maintained their contents in all the groups while PS and PE up their contents but only was statistically significant in the case of PS for squid group.

A PCA on egg lipid classes correctly characterized the three groups of eggs, extracting two principal components (which represented 70% of the total variability) related to PE, PC, CHO, TG (factor 1) and PI (factor 2) (Fig. 2). Scattering diagram achieved of this PCA was able to separate the sardine group of eggs as compared to squid and crab ones. Similarly to the eggs, PCA of the lipid classes of the hatchlings showed a tendency to separate the sardine group to the squid group. In this analysis, factor 1 (48%) was mainly correlated to the phospholipids PC and PE, while factor 2 (18%) was correlated to SM, EE and TG (Fig. 2).

Total lipid fatty acid contents of the eggs and hatchlings are presented in Table 7. Regardless of the diet supplied to the broodstocks, a predominance of the saturates and n-3 HUFA groups was observed, amounting to a 63–75% of the total FA content.

Despite of considerable within-group variability some general trends are: i) higher total content of n-6 FA in eggs and hatchlings of the crab group, mainly due to AA and 22:5n-6, and ii) higher (not significant) n-3 HUFA content in eggs and hatchlings of the squid group, mainly due to DHA. As for FA ratios: i) n-3/n-6 and AA/EPA for eggs and hatchlings reflected the higher content of n-6 series in crab group, ii) this group showed a lower EPA/DHA than sardine group, and iii) the highest 18:1n-9/n-3 HUFA ratio for eggs was found in the sardine group.

During the embryonic development, AA dropped sharply (50–51%, P < 0.07) in all dietary groups, while total FA of n-6 series decreased 44, 36 and 19% in the crab, squid and sardine groups respectively (data not showed). n-3 HUFA catabolism showed few between-group differences, amounting to 14%, 20% and 15% for squid, crab and sardine groups respectively. DHA variation was not associated to broodstock diet either, showing a relatively low and not significant reduction (19–23%). In contrast, EPA maintained similar contents in eggs and hatchlings. In general terms, saturated and monoenes were significantly consumed in crab (37–42%) and sardine (38–48%) groups but not in the squid group (11%). 18:0 is an exception with an increment in all dietary groups.

Table 6
Moisture (%), total protein, total lipid, total polar lipids, total neutral lipids (μg/indiv.) and lipid classes (ng/indiv.) contents of the eggs (embryonic stage I-IV) and hatchlings from *O. vulgaris* broodstock fed with squid, crab or sardine.

	Eggs I–IV			Hatchling		
	Squid	Crab	Sardine	Squid	Crab	Sardine
Moisture	63.4 ± 2.0	63.7 ± 2.7	66.5 ± 3.9	77.0 ± 6.0	81.2 ± 5.0	83.9 ± 6.5
Total protein	321 ± 32	279 ± 10	250 ± 41	159 ± 16	161 ± 21	128 ± 22
Total lipid	30.4 ± 7.4	29.7 ± 2.1	23.8 ± 2.2	26.9 ± 1.2	22.6 ± 5.8	16.7 ± 4.6
Total polar lipids	18.2 ± 4.4	17.9 ± 1.3	13.8 ± 1.4	17.1 ± 0.5	14.8 ± 3.7	10.8 ± 3.6
Total neutral lipids	12.3 ± 3.0	11.9 ± 0.8	10.0 ± 0.8	9.9 ± 1.3	7.9 ± 2.3	6.0 ± 1.0
Lysophosphatidylcholine	81 ± 3	103 ± 14	178 ± 55			
Sphingomyelin	217 ± 32b	287 ± 13 a	156 ± 33b	271 ± 98	192 ± 19	216 ± 93
Phosphatidylcholine	13,355 ± 3423	12,935 ± 902	9320 ± 968	9308 ± 1045a	7357 ± 1736ab	5349 ± 1760b
Phosphatidylserine	387 ± 314	431 ± 104	470 ± 150	1597 ± 684	1932 ± 829	1224 ± 498
Phosphatidylinositol	499 ± 174	520 ± 23	650 ± 211	909 ± 319	885 ± 478	410 ± 132
Phosphatidylethanolamine	3644 ± 876	3588 ± 246	3048 ± 233	4994 ± 413	4395 ± 1164	3556 ± 1214
Cholesterol	6531 ± 1550	6359 ± 446	4778 ± 123	6033 ± 392	4503 ± 1283	4317 ± 1221
Free fatty acids				452 ± 249	476 ± 407	588 ± 374
Triacylglycerol	3115 ± 726	3495 ± 326	2848 ± 812	1742 ± 945	1779 ± 386	584 ± 123
Sterol ester	2611 ± 789	2023 ± 335	2328 ± 22	1636 ± 741	1105 ± 454	505 ± 471

Results represent means (n = 3) ± standard deviation (SD). Different letters in the same row mean significant differences (P < 0.05) within the stage.

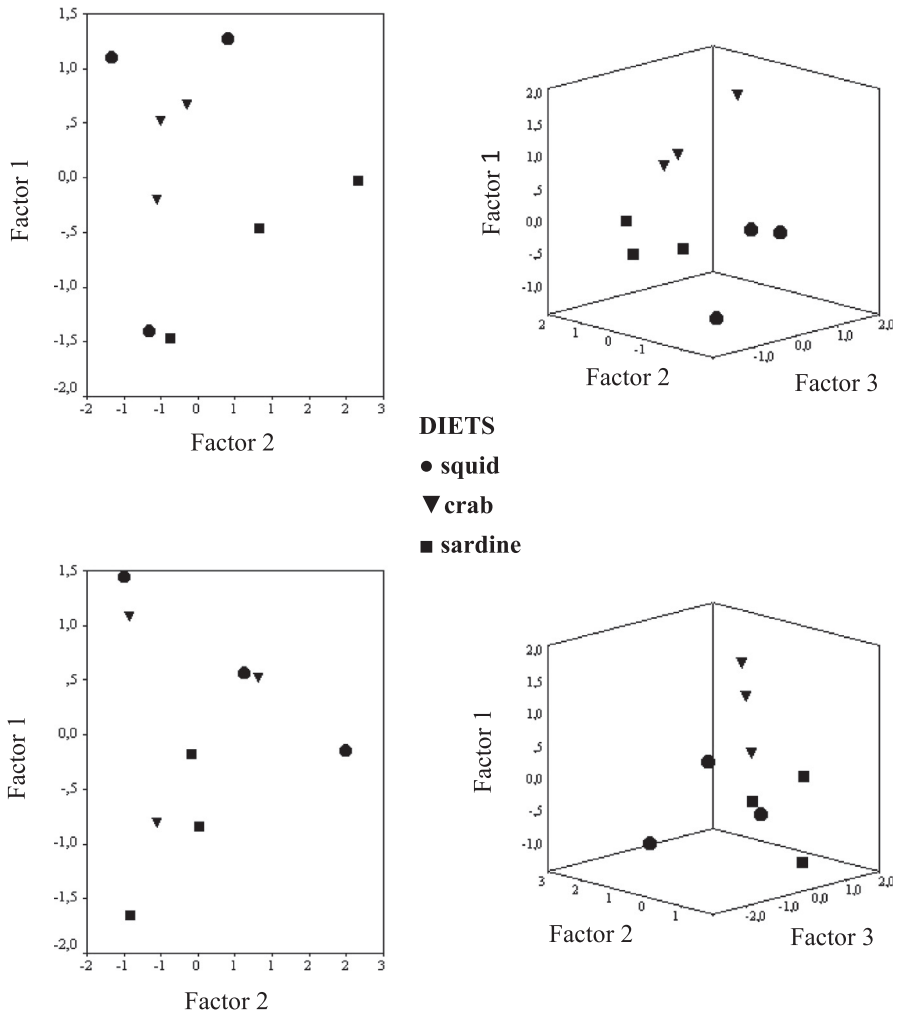

Fig. 2. Distribution of *O. vulgaris* egg (stages I–IV) (up) and hatchlings (down) samples obtained from broodstock fed the three diets in the axes obtained from principal component analyses of their lipid class content (left) and total lipid fatty acid composition (right).

PCA of total lipid fatty acids of eggs represented 75% of the total variability, extracting three principal components (Fig. 2). Factor 1 (42%) was related to n-6 HUFA, mainly AA, and saturates 15:0, 17:0 and 18:0. This factor was able to separate the crab group from squid and sardine groups. Factor 2 (19%) was related to monoenes (mostly n-9 series and 14:1n-5 and 16:1n-5) and saturates like 14:0 and 20:0. Factor 3 (13%) was mainly related to EPA and DHA. The last two axes were able to separate the squid and sardine groups between themselves.

Fig. 3(A) represents the total lipid FA composition of eggs in their two discriminant functions, by means of a scattering diagram. 97.8% and 2.2% of the total variability was explained by the discriminant functions 1 and 2, respectively, which clearly separated the three groups of eggs according to the parental diet. Using these functions, 100% of original grouped cases were correctly classified.

PCA of the total lipid fatty acids of the hatchlings is represented in Fig. 2. In a similar way to the eggs, factor 1 (36%) was highly correlated to n-6 HUFA, mainly AA, saturates 15:0 and 17:0 and monoenes 16:1, 16:1n-7 and 18:1n-7. This factor showed a tendency to separate the hatchlings of the crab group from the other groups. Factor 2 (27%) was associated to the remaining monoenes (mostly n-9 series and 16:1n-5). Factor 3 (20%) was related to saturates (mostly 16:0 and 14:0), 16:3n-3, EPA and DHA. These two last factors tend to separate hatchlings of the squid and sardine groups.

Analysis of the total lipid fatty acid composition of the hatchlings using DA, achieved that function 1 explained a 96.7% of the total variability while function 2 explained a 3.3% (Fig. 3(B)). The first function kept apart the hatchling samples from the squid treatment while the second function distinguished the sardine group from the others, thus 100% of original grouped cases were correctly classified.

3.3. Relationship between spawn-quality measures and offspring biochemical composition

Significant and positive correlations were found between the egg TL content (μg/ind) and biometrical parameters (Table 8) such as W10 ($P < 0.025$), MVL ($P < 0.05$) and L10 ($P < 0.05$). Hatchling TL content (μg/ind) was strongly correlated to EWW, HDW and L10 ($P < 0.025$) as well as W10 and L20 ($P < 0.05$). Egg TL expressed as dry weight percentage (data not shown) was negative and significantly correlated with S3 and T75% of starved hatchlings, while hatchling TL showed not significant correlations.

On the other hand, egg TP content was positively correlated to the most part of the biometrical parameters (Table 8): HDW, MVL and L10 ($P < 0.025$) and EWW, W10 and L20 ($P < 0.05$). Hatchling TP content was correlated with EWW, HDW, L10 and T100 ($P < 0.025$) and MVL, L20, S3 and T75% ($P < 0.05$). In both stages, TP expressed as percentage, was not significantly correlated to any spawning quality parameter (data not shown).

Correlation analysis among PCA factors of the egg LC content and the spawn quality parameters showed no significant relationships

Table 7
Total fatty acid contents (ng/indiv.) of the eggs (embryonic stages I–IV) and hatchlings from *O. vulgaris* broodstock fed with squid, crab or sardine.

	Eggs I–IV			Hatchling		
	Squid	Crab	Sardine	Squid	Crab	Sardine
14:0	339 ± 128	273 ± 30	512 ± 113	182 ± 72	111 ± 21	104 ± 15
16:0	3610 ± 1460	3408 ± 303	3394 ± 639	2790 ± 618	1842 ± 415	1659 ± 405
16:1n-7	59 ± 14c	195 ± 4a	107 ± 16b	43 ± 8b	70 ± 6a	36 ± 11b
16:1n-5	104 ± 38	97 ± 5	137 ± 45	50 ± 15	36 ± 4	58 ± 16
18:0	373 ± 116b	722 ± 81a	396 ± 76b	817 ± 85	807 ± 219	697 ± 217
18:1n-9	275 ± 106b	422 ± 40b	761 ± 141a	214 ± 50	218 ± 30	266 ± 48
18:1n-7	107 ± 29b	259 ± 46a	170 ± 44ab	81 ± 13	138 ± 24	96 ± 32
20:1n-9	360 ± 111	447 ± 98	516 ± 58	395 ± 66	374 ± 75	400 ± 74
20:4n-6	318 ± 15b	1256 ± 426a	203 ± 38b	158 ± 62b	631 ± 78a	100 ± 52b
20:5n-3	1555 ± 403	1125 ± 235	1352 ± 85	1534 ± 272	1142 ± 219	1212 ± 444
22:5n-6	25 ± 12b	105 ± 29a	40 ± 10b	18 ± 7b	53 ± 10a	16 ± 14b
22:5n-3	59 ± 24b	155 ± 43a	117 ± 9ab	62 ± 11b	121 ± 30a	75 ± 25ab
22:6n-3	3479 ± 1122	2759 ± 726	2569 ± 227	2801 ± 669	2211 ± 349	1968 ± 800
UK	226 ± 165	188 ± 30	171 ± 84	178 ± 43	78 ± 24	148 ± 75
Totals[1]	11,388 ± 3451	12,407 ± 2071	11,021 ± 1211	9960 ± 1801	8573 ± 1524	7409 ± 2368
Saturates	4447 ± 1697	4662 ± 433	4423 ± 804	3941 ± 785	2924 ± 692	2584 ± 652
Monoenes	1074 ± 342b	1598 ± 170ab	1935 ± 202a	950 ± 206	992 ± 165	1013 ± 198
PUFA	5606 ± 1712	5761 ± 1524	4523 ± 365	4957 ± 1006	4623 ± 761	3811 ± 1482
n-3	5237 ± 1531	4408 ± 1017	4187 ± 316	4643 ± 973	3733 ± 657	3457 ± 1346
n-6	386 ± 180b	1530 ± 517a	297 ± 60b	246 ± 50b	824 ± 116a	239 ± 123b
n-9	685 ± 239b	913 ± 136ab	1358 ± 141a	720 ± 134	694 ± 124	764 ± 126
n-3 HUFA	5110 ± 1549	4106 ± 997	4075 ± 327	4417 ± 945	3492 ± 600	3266 ± 1276
n-3/n-6	14.6 ± 5.1a	3.0 ± 0.4b	14.4 ± 2.1a	18.9 ± 2.1a	4.5 ± 0.4b	15.6 ± 3.3a
EPA/DHA	0.45 ± 0.03ab	0.41 ± 0.03b	0.53 ± 0.02a	0.55 ± 0.05ab	0.51 ± 0.02b	0.63 ± 0.04a
AA/EPA	0.20 ± 0.07b	1.10 ± 0.20a	0.15 ± 0.02b	0.10 ± 0.02b	0.56 ± 0.07a	0.08 ± 0.02b
18:1/n-3H	0.05 ± 0.01b	0.11 ± 0.01b	0.19 ± 0.05a	0.05 ± 0.00	0.06 ± 0.00	0.09 ± 0.04

Results represent means (n = 3) ± standard deviation (SD). Different letters in the same row mean significant differences (P < 0.05) within the stage. UK: unknown. PUFA: polyunsaturated fatty acids. HUFA: highly unsaturated fatty acids. EPA: eicosapentaenoic acid (20:5n-3). AA: arachidonic acid (20:4n-6). DHA: docosahexaenoic acid (22:6n-3). 18:1/n-3H: ratio 18:1n-9/n-3 HUFA.
[1] Totals: include some minor components not showed.

after applying Bonferroni correction. Factor 1 (related to PE, PC, CHO and TG) was correlated to EDW (R = 0.689, P-value = 0.040) and VML (R = 0.713, P-value = 0.031); factor 2 (related with PI) was correlated with T100 (R = −0.683, P-value = 0.043). On the other hand, factors from a PCA on hatchlings LC showed positive correlations: factor 1 (associated with PE and PI) was correlated with HDW (R = 0.747, P-value = 0.021), EWW (R = 0.725, P-value = 0.027), L10 (R = 0.685, P-value = 0.042) and L20 (R = 0.700, P-value = 0.036), whereas factor 2 (associated with SM, EE and TG) correlated with W10 (R = 0.738, P-value = 0.023).

Within the correlations of the PCA factors from egg total lipid FA, factor 1 (associated with n-6 HUFA, 15:0, 17:0 and 18:0) correlated with no spawn quality variables. On the contrary, factor 2 (associated with monoenes) was negatively correlated with T100% survival (R = −0.805, P-value = 0.009), EWW (R = −0.781, P-value = 0.013) and HDW (R = −0.730, P-value = 0.026). Factor 3 (associated with DHA and EPA) correlated with W10 (R = 0.805, P-value = 0.009).

Only the factor 3 (associated with saturates, EPA and DHA) extracted from PCA on hatchling total lipid FA correlated spawn quality variables: L10 (R = 0.770, P-value = 0.015), W10 (R = 0.718, P-value = 0.029) and HDW (R = 0.700, P-value = 0.036).

4. Discussion

4.1. Effect of parental diet on offspring biometry and viability

The present study shows that feeding *O. vulgaris* broodstocks on squid and crab diets achieve superior spawn quality as compared to

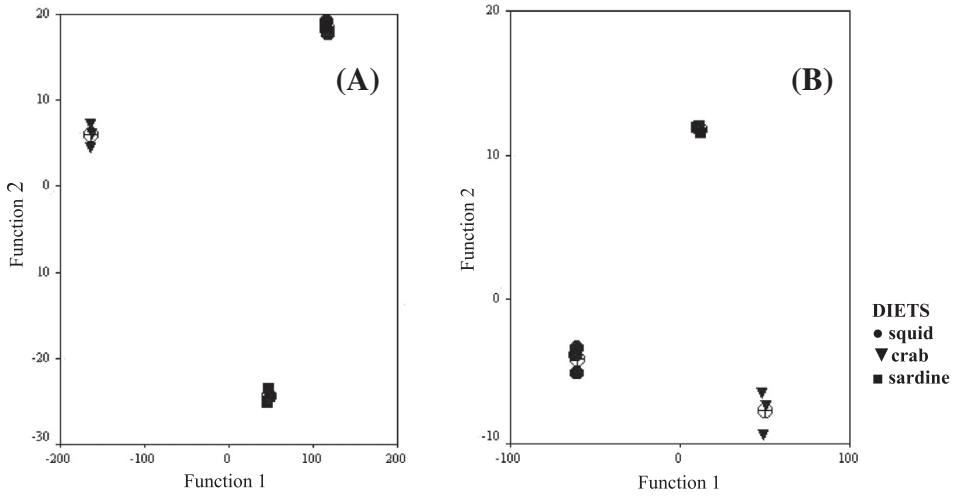

Fig. 3. Scores for the first two discriminant functions of total lipid fatty acid composition of *O. vulgaris* egg samples (stages I–IV) (A) and hatchlings (B) obtained from broodstock fed the three diet.

Table 8
Pearson correlations between spawn quality parameters and total lipid (TL) and total protein (TP) contents measured on eggs (stages I–IV) and hatchlings.

	EWW	HDW	HOC	MVL	L10	W10	L20	W20	L30	W30	S 3	T75%	T100%
Egg TL													
Pearson coef.	0.633	0.602	0.253	**0.721**	**0.692**	**0.816**	0.395	0.495	0.248	0.375	0.040	0.081	0.537
P-value	0.067	0.086	0.512	**0.029**	**0.039**	**0.007**	0.293	0.175	0.520	0.320	0.918	0.837	0.136
Egg TP													
Pearson coef.	**0.668**	**0.777**	0.170	**0.757**	**0.895**	**0.708**	**0.732**	0.417	0.294	0.484	0.173	0.281	0.561
P-value	**0.049**	**0.014**	0.661	**0.018**	**0.003**	**0.033**	**0.025**	0.265	0.443	0.187	0.655	0.463	0.116
Hatchling TL													
Pearson coef.	**0.783**	**0.841**	0.028	0.524	**0.906**	**0.671**	**0.725**	0.282	0.601	0.092	0.169	0.264	0.612
P-value	**0.012**	**0.004**	0.942	0.147	**0.001**	**0.048**	**0.027**	0.462	0.087	0.814	0.664	0.524	0.080
Hatchling TP													
Pearson coef.	**0.801**	**0.831**	0.004	0.690	0.865	0.479	**0.719**	0.218	0.589	0.017	**0.693**	**0.687**	**0.843**
P-value	**0.009**	**0.006**	0.991	**0.040**	0.003	0.192	**0.029**	0.573	0.095	0.964	**0.038**	**0.041**	**0.004**

Bold values remark significant correlations ($P < 0.05$). The measured spawn quality parameters were: egg wet weight (EWW), hatchling dry weight (HDW), hatchling organic content (HOC), hatchling mantle ventral length (MVL), 10 days-old egg length (L10), 10 days-old egg width (W10), 20 days-old egg length (L20), 20 days-old egg width (W20), 30 days-old egg length (L30), 30 days-old egg width (W30), 3 days-old hatchling survival (S3), the extinction times for 75% (T75%) and 100% (T100%) of the starved hatchlings.

sardine diet. Studies carried out in *E. megalocyathus* by Farías et al. (2011), showed that a mixed diet of frozen fish (*Odonthestes* sp.) and fresh crab (*Cancer edwarsii*) (3:1) does not improve fecundity in comparison to frozen fish alone. In this respect, the results on spawn quality herein reported are probably shaped not only by the biochemical composition of broodstock diets, but also by differences in feeding rates. Food demand per tank showed lower values for the sardine group, which was confirmed in later studies of our research group by measuring feed ingestion, as well as in other studies for the same specie (Cagneta and Sublimi, 1999). Similarly, a reduced fecundity has been observed when feed ration was diminished for the cephalopods *Idiosepius pygmaeus* (Lewis and Hodward, 1993) and *E. megalocyathus* (Farías et al., 2011), although other spawn quality indicators like egg dry weight or biochemical composition were maintained which point out to specific variations for the different spawn quality parameters.

In addition to the interspecific variations of egg size, present study has showed that broodstock diet can also influence egg biometry within a particular species. Broodstock fed sardine generally spawned smaller eggs than the squid and crab groups. This effect was reduced during embryonic development and the final lack of differences was mainly due to differences in egg swelling (Silva and Vidal, 2006), eggs from sardine group suffering a higher expansion than those from the other groups. On the other side, hatchling dry weight of the sardine group was smaller than those achieved by Navarro and Villanueva (2000) and Villanueva et al. (2004) who supplied a mixture of crab and sardine as diet for the broodstocks. On the contrary, crab and squid groups of hatchlings of the present study showed similar dry weights than those of the mentioned studies. These results suggest the adequate quality of the squid and crab and the inferior quality of sardine when used as an exclusive diet for common octopus broodstock; the use of sardine as part of mixed diets needs further studies. Nested analysis of eggs and hatchlings biometry proved a statistically significant effect of the female factor, although the associated variability was lower than that caused by the diet. The differences could be related to the genetic profile of the breeders (Benzie, 1997) as well as to environmental factors.

4.2. Egg and paralarval biometry as a predictor of paralarval viability

Data correlation analysis indicates a strong and significant relationship of egg and paralarval initial biometrical measures and final survival achieved of paralarvae under starved conditions. The sardine group presented the smaller early-staged eggs and hatchlings as well as the shorter-lived paralarvae, the opposite was true for the crab and squid groups. Since hatchling resistance to starvation is widely used as an indicator of offspring viability (Kamler, 2005), the present data suggest that egg and hatchling early biometrical measures can be utilized as a proxy for paralarval viability; those measurements being much easier to carry out than biochemical or molecular analyses. In addition, the relationship between biometrical measures and paralarvae survival suggests as well differences in the availability of stored nutrient at the hatchling time. This hypothesis and its connection to the hatchling lipid stores will be discussed in detail in the next section.

4.3. Effect of parental diet on offspring biochemical composition

The most outstanding result arising from the comparison between biochemical compositions of the diets is the high level of TL in the sardine diet, where TG represented over one half of the TL although this proportion was not significantly different from those in squid and crab diets due to the variability of the data. Such variability has been previously reported in sardine in relation to environmental or physiological factors (maturity stage, T[a], diet, etc.) (Luzia et al., 2003; Shirai et al., 2002; Zlatanos and Laskaridis, 2007). In general terms, sardine showed lower levels of relevant lipid classes such as PC, PI, CHO and EE, which could contribute to the lower quality of this diet. No differences were found between squid and crab diet with the exception of higher levels of FFA in the squid group, which could be related the high levels of this lipid class detected in the digestive gland of some cephalopods (Morillo-Velarde et al., 2012 & 2013). In a similar way to lipid classes, fatty acid composition of the diets did not show a clear separation between them, barring the higher levels of n-6 series in the crab diet.

Eggs and hatchlings obtained from different dietary groups did not show significant differences in total protein content (μg/ind.), but a tendency of higher contents in the squid group was observed corresponding to the higher level of this nutrient in the diet. On the contrary, sardine diet showed a high level of TL but the eggs from this group showed relatively lower levels of this nutrient. On the other hand, Farías et al. (2011) did not find remarkable effects of broodstock diets on the biochemical composition of eggs and paralarvae of Patagonian red octopus (*E. megalocyathus*). This disparity in the results could be explained by several factors such as nutrient profile, diet acceptance and/or digestibility/absorption.

Egg TL percentage in the present study (6.5–6.8% in a DW) was nearly half the amount in common octopus eggs from the Mediterranean coast analyzed by Navarro and Villanueva (2003) (11.0–12.0%). This dissimilarity is hardly explained by the variation in developmental stage (I–IV vs XV–XX, in the present study and Navarro and Villanueva (2003) respectively), since these authors reported a slight reduction of TL from mature ovary to the XV–XX stages (14.5% vs 11.0%). The same fact was observed in TL of hatchlings in both studies (7.4–8.5 vs 13.4%). *Sepia officinalis*' eggs from different geographical places also showed differences in the TL content (Sykes et al., 2008).

Lipid class profile of eggs and hatchlings (expressed as % TL, data not shown) was nearly constant, despite of the broodstock diet, suggesting

specific requirements during the yolk synthesis. Phospholipids and CHO were the most abundant, in agreement with data from data obtained by Navarro and Villanueva (2000) in the same species. When LC data are expressed as absolute contents (ng/indiv.) some changes were observed between eggs and hatchlings. An exploratory analysis by PCA separated the eggs from sardine group from the crab ones due to PC, PI, PE, CHO and TG whereas in hatchlings the most conspicuous difference was between squid and sardine mainly due to SM, PC, PE, EE and TG.

Egg and paralarva FA composition was characterized by high levels of saturates and n-3 HUFA, mainly due to 16:0, EPA and DHA, regardless of parental diet. These profiles match up with those observed for the same species and other cephalopod like *S. officinalis* and *Loligo vulgaris* (Navarro and Villanueva, 2000, 2003; Sykes et al., 2008). In a similar way, FA profiles of marine fish's egg are characterized by high levels of saturates and n-3 HUFA (Tocher, 2003) but, on the contrary, octopus also shows high levels of monoenes; supporting an important function of monoenes in *O. vulgaris* development. The influence of the diet on the egg and hatchling composition has been confirmed with the relatively high contents of DHA in the squid group and n-6 HUFA in the crab group; reflecting the higher levels of these fatty acids in the respective diets. A similar influence has also been observed in fish species such as *Hippoglossus hippoglossus* (Mazorra et al., 2003), *Salmo salar* (Rennie et al., 2005), *Sparus aurata* (Bell et al., 1997), *Dicentrarchus labrax* (Bruce et al., 1999) and *Paralichthys olivaceus* (Furuita et al., 2003). Recent data obtained by Monroig et al. (2012a,b) suggest that *O. vulgaris* has little or no synthesis of DHA, EPA and AA, therefore these FAs should be incorporated from the diet or from female body reserves.

The AA/EPA ratio in eggs and hatchlings of the crab group was clearly higher than those in the sardine and squid groups. Both fatty acids have an important role in the physiology of vertebrates as eicosanoid precursors (Sargent et al., 1999). However, in the present study, the mentioned differences did not seem to affect spawning quality.

4.4. Biochemical composition variation throughout the embryonic development

Regardless of the broodstock diet, compositional variations from egg to hatchling showed a higher decrease in TP (42–50%) than in TL (11–29%). This result pointed to an embryo catabolism mainly based on proteins, as previously suggested (Lee, 1994; Villanueva and Norman, 2008; Villanueva et al., 2004). Data also suggest that TG, EE and PC were specifically catabolized for energy and/or used to de novo synthesize of lipid classes such as PS or PE. As a contrast, fish species with PL-rich eggs (e.g., Atlantic herring, cod, goldfish) predominately use PC as source of energy for the embryo development (Rainuzzo et al., 1997; Wiegand, 1996). On the other hand, CHO was retained and PE and PS increased during embryogenesis, suggesting the importance of these lipid classes possibly as structural components and matching up with the lipid class composition observed in previous cephalopod studies (Navarro and Villanueva, 2000, 2003; Sykes et al., 2008).

Comparing the decrease in egg TL content among the three dietary groups, it was notable that this reduction was higher in the groups whose initial TP egg content was lower (it was smaller in the squid group than in the sardine group). The higher drop of TG and EE in the sardine group (78–79%) with respect to crab (45–49%) and squid groups (37–44%), and the initially smaller contents of TG, EE and PC, could indicate an energetic deficiency during embryogenesis which would contribute to the inferior spawn quality observed in sardine group.

During embryonic development, a general non-significant reduction of the all FA series was observed, but the change in n-3 FA was lower than that in saturates, monoenes and n-6. Within the n-6 FA, AA was strongly reduced (50–51%) during embryonic development. In contrast to LC catabolism, FA catabolism showed a relative specificity according to the broodstock diet. Saturates and monoenes decreased by 37–42% and 38–48% in sardine and crab groups respectively, but only 11% in squid group. Just like in the case of TL, the lower utilization of saturates and monoenes in the squid group could be related with a higher protein catabolism in this group. On the other side, the embryo catabolism of n-3 HUFA and n-6 series were not related to the level of protein consumption. It showed low between-group differences for n-3 HUFA (13.5%, 19.5% and 14.9% for squid, crab and sardine groups, respectively); while total FA of n-6 series decreased 44, 36 and 19% in the crab, squid and sardine groups, respectively. The higher catabolism of AA in the crab group (50%) and of 18:1n-9 in the sardine group (65%) appears to be related to the higher initial content of these FAs in the egg. Taking into account the generalized role of the AA on reproduction and spawn quality, the catabolism observed in the present study is in opposition to the selective retention observed in *Solea senegalensis*, *Maccullochella macquariensis*, *Maccullochella peelii* (Tocher, 2003) and *Diplodus sargus* (Cejas et al., 2004). Further studies on the particular role of AA for early stages of *O. vulgaris* are needed, specially taking into account that AA is an essential FA for this species (Monroig et al., 2012a,b).

Finally, the remarkable low embryonic consumption of 20:1n-9 in crab and sardine groups (16 and 22% respectively) and the low increase in squid (10%), led to a predominance of this FA in the monoene series of the hatchlings from all the dietary groups. High levels of this FA has been also found in previous studies in *O. vulgaris* (Navarro and Villanueva, 2003; Reis et al., 2014), so that further studies are necessary to figure out the reason for its conservation.

4.5. Relationship between spawn-quality measures and offspring biochemical composition

The present study shows that TP and TL contents of eggs and hatchlings were correlated to the main biometrical parameters measured at both stages (EDW, L10, W10, HDW, MVL) (Table 8). Since dietary treatments exerted a significant effect on spawn quality, correlations between both spawn quality and composition of macronutrients could be caused by the diet supplied to the broodstocks.

Results also showed a correlation of the measured survival (S3, T75% and T100%) with the TP content of hatchlings but not with their TL content. These data highlighted the importance of protein reserves at the beginning of the exogenous feeding of paralarvae.

The lipid class composition of eggs and hatchlings (represented by means of the respective PCA axes) has also shown strong and significant correlations with the spawn quality parameters (data not shown). This analysis also suggests that the lipid classes with higher levels (PE, PC, CHO and TG) explained the main effect on the spawn quality. These results could altogether indicate that the broodstock diet had an influence on the variations of the LC as well as the spawn quality parameters and both of them were found to be significantly correlated.

Saturates, monoenes and AA were the FA with a higher loading factor for between groups separation of eggs and hatchlings by PCA. Spawn quality parameters were mainly correlated to the third axis of the PCA, which was associated with EPA and DHA (data not shown). A similar relationship between n-3 HUFA and spawn quality has been reported for different marine species (Bo Wen et al., 2002; Furuita et al., 2002; Li et al., 2005; Mazorra et al., 2003). If DHA is required for the visual system as in fish larvae (Benítez-Santana et al., 2007), it can be important for the visually-driven predatory activity of common octopus paralarvae (Márquez et al., 2007).

In addition, a significant negative correlation between the hatchling survival indicators and the monoene levels of eggs and hatchlings (factor 2 of the fatty acids PCA) were found, suggesting that high monoene levels in the yolk could be unfavorable for the paralarval viability as happens in sardine group of eggs. Therefore, the level of monoenes or the n-3 HUFA/monoene ratio could be suggested as another compositional indicator of the spawn quality in common octopus.

In conclusion, our results suggest that the broodstock diet influenced the protein content and lipid composition of eggs and hatchlings, and these nutrients are related to the differences found in the spawn quality, though differences in food ingestion and digestibility deserve further investigation.

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References

A.O.A.C., 1995. Official Methods of Analysis of the Association of Analytical Chemistry, Washington D.C., U.S.A. (1018 pp.).

Aby-ayad, S.-M.-A., Melard, C., Kestemont, P., 1997. Effects of fatty acids in Eurasian perch broodstock diet on egg fatty acid composition and larvae stress resistance. Aquac. Int. 5, 161–168.

Almansa, E., Pérez, M.J., Cejas, J.R., Badía, P., Villamandos, J.R., Lorenzo, A., 1999. Influence of broodstock Gilthead seabream (*Sparus aurata* L.) dietary fatty acids on egg quality and egg fatty acid composition throughout the spawning season. Aquaculture 170, 323–336.

Bell, J.G., Farndale, B.M., Bruce, M.P., Navas, J.M., Carrillo, M., 1997. Effects of broodstock dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax*). Aquaculture 149, 107–119.

Benítez-Santana, T., Masuda, R., Juárez Carrillo, E., Ganuza, E., Valencia, A., Hernández-Cruz, C.M.A., Izquierdo, M.S., 2007. Dietary n-3 HUFA deficiency induces a reduced visual response in gilthead seabream *Sparus aurata* larvae. Aquaculture 264, 408–417.

Benzie, 1997. A review of the effect of genetics and environment on the maturation and larval quality of the giant tiger prawn *Penaeus monodon*. Aquaculture 155, 69–85.

Bo Wen, X., Qiao Chen, L., Liang Zhou, Z., Xiang Ai, C., Deng, G., 2002. Reproduction response of Chinese mitten-handed crab (*Eriocheir sinensis*) fed different sources of dietary lipid. Comp. Biochem. Physiol. A 131, 675–681.

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

Bruce, M., Oyen, F., Bell, G., Asturiano, J., 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.

Cagneta, P., Sublimi, A., 1999. Productive performance of the common octopus (*Octopus vulgaris* C.) when fed on a monodiet. Seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterraean on "Recent Advances in Mediterranean Aquaculture Finfish Species Diversification".

Carrasco, J.L., 1995. El método estadístico en la investigación médica. Ciencia 3 (Madrid).

Cejas, J.R., Almansa, E., Jérez, S., Bolaños, 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. Physiol. B 139, 209–216.

Christie, W.W., 1982. Lipids Analysis. 2nd edn. Pergamon Press, Oxford.

Farías, A., Navarro, J.C., Cerna, V., Pino, C., Uriarte, I., 2011. Effect of broodstock diet on the fecundity and biochemical composition of eggs of the Patagonian red octopus (*Enteroctopus megalocyathus* Gould 1852). Cienc. Mar. 37 (1), 11–21.

Fernández-Palacios, H., Izquierdo, M., Robaina, L., Valencia, A., Salhi, M., Montero, D., 1997. The effect of dietary protein and lipid from squid and fish meals on egg quality of broodstock for gilthead seabream (*Sparus aurata*). Aquaculture 148, 233–246.

Fowler, J., Cohen, L., Jarvis, P., 1998. Practical Statistics for Field Biology. John Wiley and sons Ltd, West Sussex,England (259 pp.).

Fukazawa, H., Kawamura, T., Takami, H., Watanabe, Y., 2007. Oogenesis and relevant changes in egg quality of abalone *Haliotis discus hannai* during a single spawning season. Aquaculture 270, 265–275.

Furuita, H., Tanaka, H., Yamamoto, T., Shiraishi, M., Takeuchi, T., 2000. Effects of n-3 HUFA levels in broodstock diet on the reproductive performance and egg and larval quality of the Japanese flounder, *Paralichthys olivaceus*. Aquaculture 187, 387–398.

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.

George, S.B., 1999. Egg quality, larval growth and phenotypic plasticity in a forcipulate seastar. J. Exp. Mar. Biol. Ecol. 237, 203–224.

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

Iglesias, P., Fuentes, L., 2014. Research on the production of the hatchery-reared juveniles of cephalopods with special reference to the common octopus (*Octopus vulgaris*). In: Allan, G., Burnell, G. (Eds.), Advances in Aquaculture Hatchery Technology. Woodhead publishing, Cambridge, pp. 375–403.

Iglesias, J., Sánchez, F.J., Bersano, J.G.F., Carrasco, J.F., Dhont, J., Fuentes, L., Linares, F., Muñoz, J.L., Okumura, S., Roo, J., Van der Meeren, T., Vidal, E.A.G., Villanueva, R., 2007. Rearing of *Octopus vulgaris* paralarvae: present status, bottlenecks and trends. Aquaculture 266, 1–15.

Iglesias, P., Pazos, G., Fernández, J., Sánchez, F.J., Otero, J.J., Domingues, P., Lago, M.J., Linares, F., 2013. The effects of using crab zoeae (*Maja brachydactyla*) on growth and biochemical composition of *Octopus vulgaris* (Cuvier 1797) paralarvae. Aquac. Int. http://dx.doi.org/10.1007/s10499-013-9725-7.

Izquierdo, M., Fernández-Palacios, H., Tacon, A., 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197, 25–42.

Kamler, E., 2005. Parent–egg–progeny relationships in teleost fishes: an energetics perspective. Rev. Fish Biol. Fish. 15, 399–421.

Kjorsvik, E., Mangor-Jesen, A., Holmefjord, I., 1990. Egg quality in fishes. Adv. Mar. Biol. 26, 71–113.

Lee, P.G., 1994. Nutrition of cephalopods: fuelling the system. Mar. Freshw. Behav. Physiol. 25, 35–51.

Lewis, A.R., Hodward, J., 1993. Spawning mode and reproductive output of the tropical cephalopod *Idiosepius pygmaeus*. Can. J. Fish. Aquat. Sci. 50, 20–28.

Li, Y., Chen, W., Sun, Z., Chen, J., Wu, K., 2005. Effects of n-3 HUFA content in broodstock diet on spawning performance and fatty acid composition of eggs and larvae in *Plectorhynchus cinctus*. Aquaculture 245, 263–272.

Luzia, L.A., Sampaio, G.R., Castellucci, C.M.N., Torres, E.A.F.S., 2003. The influence of season on the lipid profiles of five commercially important species of Brazilian fish. Food Chem. 83, 93–97.

Márquez, L., Quintana, D., Almansa, E., Navas, J.I., 2007. Effects of visual conditions and prey density on feeding kinetics of paralarvae of *Octopus vulgaris* from a laboratory spawning. J. Molluscan Stud. 73, 117–121.

Márquez, L., Quintana, D., Lorenzo, A., Almansa, E., 2013. Biometrical relationships in developing eggs and neonates of *Octopus vulgaris* in relation to parental diet. Helgol. Mar. Res. 67, 461–470.

Mazorra, C., Bruce, M., Bell, J.G., Davie, A., Alorend, E., Jordan, N., Rees, J., Papanikos, N., Porter, M., Bromage, N., 2003. Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 227, 21–33.

Migaud, H., Bell, G., Cabrita, E., McAndrew, B., Davie, A., Bobe, J., Herráez, M.P., Carrillo, M., 2013. Gamete quality and broodstock management in temperate fish. Rev. Aquac. 5, 194–223.

Monroig, O., Guinot, D., Hontoria, F., Tocher, D.R., Navarro, J.C., 2012a. Biosynthesis of essential fatty acids in *Octopus vulgaris* (Cuvier, 1797): molecular cloning, functional characterisation and tissue distribution of a fatty acyl elongase. Aquaculture 360–361, 45–53.

Monroig, O., Varó, I., Tocher, D.R., Navarro, J.C., 2012b. Isolation and functional characterisation of a stearoyl-CoA desaturase from the marine invertebrate *Octopus vulgaris*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 163, 46–47.

Morillo-Velarde, P.S., Cerezo, Valverde J., Hernández, M.D., Aguado-Giménez, F., García, García B., 2012. Growth and digestibility of formulated diets based on dry and freeze–dried ingredients in the common octopus (*Octopus vulgaris*). Aquaculture 368–369, 139–144.

Morillo-Velarde, P.S., Cerezo, Valverde J., Serra Llinares, R.M., García, García B., 2013. Changes in lipid composition of different tissues of common octopus (*Octopus vulgaris*) during short-term starvation. Aquac. Res. 44 (8), 1177–1189.

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.

Muñoz Serrano, A., 2003. Estadística aplicada uni y multivariante. Conserjería de Agricultura y Pesca de la Junta de Andalucía (Ed), Sevilla, Spain, 1018 pp.

Navarro, J.C., Villanueva, R., 2000. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. Aquaculture 183, 161–177.

Navarro, J.C., Villanueva, R., 2003. The fatty acid composition of *Octopus vulgaris* paralarvae reared with live and inert food: deviation from their natural fatty acid profile. Aquaculture 219, 613–631.

Navarro, J.C., Monroig, O., Sykes, A.V., 2014. Nutrition as a key factor for cephalopod aquaculture. In: Iglesias, J., Fuentes, L., Villanueva, R. (Eds.), Cephalopod, Culture, pp. 77–95.

Olsen, R.E., Henderson, R.J., 1989. The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. J. Exp. Mar. Biol. Ecol. 129, 189–197.

Ostrowski, A.C., Divakaran, S., 1991. Energy substrates for eggs and prefeeding larvae of the dolphin *Coryphaena hippurus*. Mar. Biol. 109, 149–155.

Politis, S.N., Dahlke, F.T., Butts, I.A.E., Peck, M.A., Trippel, E.A., 2014. Temperature, paternity and asynchronous hatching influence early developmental characteristics of larval Atlantic cod, *Gadus morhua*. J. Exp. Mar. Biol. Ecol. 459, 70–79.

Quintana, D., Márquez, L., Almansa, E., Bolaños, A., Lorenzo, A., 2006. Efecto de la dieta de reproductores de pulpo común (*Octopus vulgaris*) en la composición bioquímica de paralarvas bajo condiciones de inanición. In: Cruz Suárez, L.E., Ricque Marie, D., Nieto López, M.G., Tapia Salazar, M., Villarreal Cavazos, D., Puello Cruz, A.C., y García Ortega, A. (Eds.), Avances en nutrición acuícola VIII. Memorias del VIII Simposio Internacional de Nutrición Acuícola. 15 al 17 de Noviembre de 2006. Mazatlán, Sinaloa, México. Universidad Autónoma de Nuevo León, Monterrey, N.L., México ISBN 970-694-331-5.

Quintana, D., Domingues, P., García, S., 2008. Effect of two artificial wet diets agglutinated with gelatin on feed and growth performance of common octopus (*Octopus vulgaris*) sub-adults. Aquaculture 280 (1–4), 161–164.

Rainuzzo, J., Reitan, K., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155, 103–115.

Rainuzzo, J.R., 1993. Lipids in Early Stages of Marine Fish. (PhD Thesis). University of Trondheim, Norway.
Reis, D.B., Acosta, N.G., Almansa, E., Navarro, J.C., Tocher, D.R., Monroig, O., Andrade, J.P., Sykes, A.V., Rodríguez, C., 2014. In vivo metabolism of unsaturated fatty acids in *Octopus vulgaris* hatchlings determined by incubation with 14C-labelled fatty acids added directly to seawater as protein complexes. Aquaculture 431, 28–33.
Rennie, S., Huntingford, F.A., Loeland, A., Rimbach, M., 2005. Long term partial replacement of dietary fish oil with rapeseed oil; effects on egg quality of Atlantic salmon *Salmo salar*. Aquaculture 248, 135–146.
Ruohonenen, K., 1998. Individual measurements and nested designs in aquaculture experiments: a simulation study. Aquaculture 165, 149–157.
Sargent, J.R., Bell, M.V., Bell, J.G., Henderson, R.J., Tocher, D.R., 1995. Origin and functions of n-3 polyunsaturated fatty acids in marine organisms. In: Cevc, G., Paltauf, F. (Eds.), Phospholipids: Characterisation, Metabolism and Novel Biological Applications. American Oil Chemist Society Press, Champaign, Ill, USA, pp. 248–258.
Sargent, J.R., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D.R., 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179, 217–229.
Shirai, N., Terayama, M.a., Takeda, H., 2002. Effect of season on the fatty acid composition and free amino acid content of the sardine *Sardinops melanostictus*. Comp. Biochem. Physiol. B 131, 387–393.
Silva, L., Vidal, E., 2006. Yolk utilization, water, organic and inorganic content of *Octopus vulgaris* eggs during embryonic development. Cephalopod International Advisory Council Symposium (6–10 February, Hobart, Tasmania).
Steer, M.A., Moltschaniwskyj, N.A., Nichols, D.S., Miller, M., 2004. The role of temperature and maternal ration in embryo survival: using the dumpling squid *Euprymna tasmanica* as a model. J. Exp. Mar. Biol. Ecol. 307, 73–89.
Sykes, A.V., Almansa, E., Lorenzo, A., Andrade, J.P., 2008. Lipid characterization of both wild and cultured eggs of cuttlefish (*Sepia officinalis* L.) throughout the embryonic development. Aquac. Nutr. 15 (1), 38–53.
Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107–184.
Vaz-Pires, P., Seixas, P., Barbosa, A., 2004. Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. Aquaculture 238, 221–238.
Villanueva, R., Norman, M.D., 2008. Biology of the planktonic stages of benthic octopuses. Oceanogr. Mar. Biol. Annu. Rev. 46, 105–202.
Villanueva, R., Riba, J., Ruíz-Capillas, C., Gonzalez, A.V., Baeta, M., 2004. Aminoacid composition of early stages of cephalopods and effect of aminoacid dietary treatments on *Octopus vulgaris* paralarvae. Aquaculture 242 (1–4), 455–478.
Watanabe, T., Vasallo-Agius, R., 2003. Broodstock nutrition research on marine finfish in Japan. Aquaculture 227 (1–4), 35–61.
Wiegand, M.D., 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. Rev. Fish Biol. Fish. 6, 259–286.
Zar, J.H., 1999. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, New Jersey (663 pp.).
Zlatanos, S., Laskaridis, 2007. Seasonal variation in the fatty acid composition of three Mediterranean fish — sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*). Food Chem. 103, 725–728.