Aquaculture Nutrition

Aquaculture Nutrition 2014 doi: 10.1111/anu.12205

An insight on *Octopus vulgaris* paralarvae lipid requirements under rearing conditions

D.B. REIS[1,2,3], I. GARCÍA-HERRERO[1,2], R. RIERA[4], B.C. FELIPE[1], C. RODRÍGUEZ[2], A.V. SYKES[3], M.V. MARTÍN[1], J.P. ANDRADE[3] & E. ALMANSA[1]

[1] *Instituto Español de Oceanografía, Centro Oceanográfico de Canarias, Santa Cruz de Tenerife, Spain;* [2] *Departamento de Biología Animal, Edafología y Geología, Universidad de La Laguna, La Laguna, Santa Cruz de Tenerife, Spain;* [3] *CCMAR-CIMAR L.A., Centro de Ciências do Mar do Algarve, Universidade do Algarve, Faro, Portugal;* [4] *Centro de Investigaciones Medioambientales del Atlántico (CIMA SL), La Laguna, Santa Cruz de Tenerife, Spain*

Abstract

In this study, two new alternative preys: *Grapsus adscensionis* zoeae (as sole prey) and *Palaemon elegans* zoeae (in cofeeding with *Artemia* sp.), as well as, *Artemia* sp. juveniles were used as feed for octopus paralarvae, as a way to understand its lipid requirements. Total lipid (TL) content, lipid class (LC) and fatty acid (FA) profiles of preys, octopus hatchlings and 9-day-old paralarvae were analysed. Growth and survival of the paralarvae were also determined. Regardless the prey provided, a notable shift in the lipid profile of paralarvae was registered after 9 days of rearing. The highest index of growth rate (IGR) recorded when decapod crustacean zoeae were supplied might have some relation with levels of 20:4n-6 (ARA) and DHA/EPA ratio observed. In this sense, *Grapsus adscensionis* zoeae leaded to a higher content of ARA and a lower content of EPA, which may indicate a possible competition between these two FA. For that a balanced EPA/ARA ratio might be significant in this species nutrition without disconsidering DHA levels as an essential fatty acid. Finally, the changes observed in paralarvae FA profile might not only be related to prey FA profile, but also with changes occurring in the lipid classes contents.

KEY WORDS: *Artemia* sp. juveniles, decapod crustacean zoeae, lipid requirements, *Octopus vulgaris*, paralarval rearing

Received 16 October 2013; accepted 15 April 2014

Correspondence: D.B. Reis, Departamento de Biología Animal, Edafología y Geología, Facultad de Biología, Universidad de La Laguna, 38206 Tenerife, Spain. E-mail: dfbreis@ualg.pt

Introduction

The common octopus (*Octopus vulgaris*) has been pointed as a candidate species for aquaculture diversification due to its short life cycle, rapid growth and high food conversion index (Vaz-Pires *et al.* 2004). However, the rearing of the planktonic stage still remains the main bottleneck in the culture of this species, due to high mortality rates verified during this life stage (Iglesias *et al.* 2007; Villanueva & Norman 2008).

In a similar way to what has been reported to other cephalopod species [e.g. cuttlefish, Sykes *et al.* (2006)], the choice of a particular prey which will meet the nutritional requirements for the metabolism of octopus, paralarvae will be a key factor to overcome the problem of rearing octopus hatchlings through this stage (Iglesias *et al.* 2007). *Octopus vulgaris* is a carnivorous species and an opportunistic predator that, throughout its life cycle and depending on seasonal and geographical variations, feeds on a wide variety of preys, such as crustaceans, fishes, bivalves and other cephalopods (Mangold 1983; Hanlon & Messenger 1996). According to Roura *et al.* (2012), the planktonic paralarvae can predate on more than 12 different families of crustaceans. In this sense, some crustacean species have been tried as prey for *O. vulgaris* paralarvae rearing (Itami *et al.* 1963; Villanueva 1994, 1995; Moxica *et al.* 2002; Carrasco *et al.* 2006; Iglesias *et al.* 2013). The life cycle of *O. vulgaris* under captivity was completed for the first time using spider crab (*Maja squinado*) zoeae as prey in cofeeding with *Artemia* sp. (Iglesias *et al.* 2004). Nonetheless, and despite of high growth rates and benthic stage being accomplished, these experiments have resulted in very low survival rates. In addition, *Maja* zoeae are hard to rear, highly expensive to obtain and its juveniles and adults

display higher commercial value than the common octopus. According to Iglesias *et al*. (2007), the difficulty of logistics on provision of zooplankton to octopus paralarvae makes necessary the use of a prey with wide availability and acceptance by paralarvae such as *Artemia* sp. However, the high mortalities obtained in *O. vulgaris* paralarvae rearing with *Artemia* sp., have been associated with a nutritional imbalance of its lipid profile (Navarro & Villanueva 2000, 2003; Iglesias *et al*. 2007; Seixas *et al*. 2010a,b). Paralarvae require a prey rich in polyunsaturated fatty acids (PUFA), phospholipids and cholesterol (Navarro & Villanueva 2000, 2003). However, *Artemia* sp. presents a low content in PUFA, such as 20:5n-3 (EPA), and is particularly absent on 22:6n-3 (DHA) (Navarro & Villanueva 2000). Therefore, an improvement in *Artemia* sp. lipid composition seems necessary. Several studies have described the effects of *Artemia* sp. enrichment on paralarval growth and survival, but those results are still far away from the obtained with crustacean zoeae (Navarro & Villanueva 2000, 2003; Seixas *et al*. 2010a,b; Fuentes *et al*. 2011; Viciano *et al*. 2011). Other aspect to be taken into account is the role of the lipid classes of the prey in octopus paralarval development. This knowledge is still very scarce, with few studies analysing this aspect (Navarro & Villanueva 2000 Moxica *et al*. 2002; Iglesias *et al*. 2013), being Navarro & Villanueva (2000) the only study presenting a complete characterization of lipid classes, including polar lipid composition. In this sense, it is necessary to improve the knowledge on lipid requirements of *O. vulgaris* paralarvae, which could be achieved using different crustacean zoeae as prey and studying their lipid class and fatty acids composition and effects on paralarvae lipid profiles and corresponding growth and survival rates at early paralarvae stages. Nowadays these studies are scarce, only focused on *Maja* zoeae and always in cofeeding with *Artemia* sp. (Moxica *et al*. 2002; Iglesias *et al*. 2013). Therefore, the use of alternative crustacean species, such as *Palaemon elegans* and *Grapsus adscensionis*, as prey models could improve our present knowledge in paralarvae lipid dynamics. In particularly, *G. adscensionis* shows a high fecundity and is of easy culture of (Scherbakova *et al*. 2011) which could allow for the first time, the use of crustacean zoeae as sole prey for paralarvae feeding.

The objective of the present study was to assess the *O. vulgaris* paralarvae lipid requirements, under rearing conditions, by a complete characterization of its lipid composition profile (fatty acid and lipid classes composition) at hatching and after 9 days of feeding with three different live preys: *Artemia* sp. juveniles, *G. adscensionis* zoeae, as sole prey; or a cofeeding of *Artemia* sp. with *Palaemon elegans* zoeae. The effect of those preys on paralarvae growth and survival after 9 days of rearing was also determined.

Material and methods

Octopus vulgaris broodstock and eggs

Octopus vulgaris broodstock individuals were caught by professional artisanal fishermen on Tenerife coast (Canary Islands, Spain). Individuals with a mean wet weight (MWW) of 1309 ± 503 g, were kept in 1000 L circular fibreglass tanks of a flow-through seawater system, under natural photoperiod (from 10L : 14D to 11L : 13D h of light and dark), water temperature of 21.01 ± 0.69 °C and 36.8 ± 0.14 g L^{-1} of salinity. Temperature was measured with a Tinytag Plus 2 (TGP-4020; Gemini Data Loggers Ltd., Chichester, West Sussex, UK) and salinity with a Refractometer S/Mill-E (ATAGO, Tokyo, Japan). Three octopuses with similar MWW were placed in each tank, establishing a sex ratio of two females per male (2♀ : 1♂), which contained two pots per octopus as shelter, to avoid shelter competition. Individuals were fed *ad libitum* on frozen squid (*Loligo opalescens*). The presence of eggs was verified once a week and, when egg masses were observed in the pot, the ovate female was left alone in the tank by removing the other octopuses.

Experimental setup and sample collection

Octopus hatchlings, with a mean size of 1.20 ± 0.05 mm [ventral mantle length (VML)] and a mean dry weight (DW) of 0.17 ± 0.02 mg, were reared in 100 L fibreglass cylinder conical tanks (52 cm of diameter and 56 cm from top to bottom) of a flow-through seawater system with a 60 mL min^{-1} water flow, at a density of 1.5 paralarvae L^{-1} (150 individuals per tank). A light intensity of 200 lx (provided by an incandescent 40 W bulb) and a photoperiod of 12L : 12D (light from 8:00 a.m. to 8:00 p.m.) were maintained throughout the experiment. Green water technique was applied to all rearing tanks, daily adding 200 000 cells mL^{-1} of *Chlorella* spp. to each tank, prior lights were turned on. The mean water temperature of the rearing tanks was 22.42 ± 0.26 °C, salinity was 36.8 ± 0.14 g L^{-1} and dissolved oxygen was near saturation. Nitrogenous compound build-up was assessed at the end of the experiment with the use of TETRA test aquarium kits. NH_3, NO_2 and NO_3 values were 0–0.25 ppm,

<0.3 ppm and 0–1.25 ppm, respectively. In addition, pH was also daily verified with a Hanna-HI-98107 pH Metter and displayed values within 7.9–8.0.

Three different paralarvae groups based on diets were established: ART – fed on *Artemia* sp. juveniles; PAL : ART – a cofeeding of *P. elegans* zoea and *Artemia* sp.; and GRA – fed on *G. adcensionis* zoea. The use of cofeeding in PAL : ART was justified by the lack of enough *P. elegans* zoeas. Feeding densities were as follows for each diet treatment: ART – 0.03–0.07 artemia mL^{-1}; PAL : ART – 0.001–0.006 zoea mL^{-1}: 0.03–0.07 artemia mL^{-1} (in a ratio of 3 zoea per 50 artemia juveniles) and GRA – 0.01 zoea mL^{-1}. Every diet treatment was assessed in quadruplicate, and all tanks were fed *ad libitum*. In a way to ensure both a proper amount of sample for biochemical composition analyses and growth rates determination, without compromising the biochemical effect of preys over paralarvae lipid profile visualization, rearing experiments were finished at day nine.

Fifteen individuals of *O. vulgaris* hatchlings and 9-day-old paralarvae from each replicate tank were collected to calculate: (i) dry weight (DW; mg); (ii) ventral mantle length (VML; mm); (iii) instantaneous growth rate (IGR;% DW t^{-1}) = $(\mathrm{Ln}W_2 - \mathrm{Ln}W_1)\ t^{-1}\cdot 100$, where W_1 and W_2 are the initial and final dry weight, respectively, Ln the natural logarithm and t the number of days of the experiment. Also survival rate (SR;%) was determined as $(100\cdot N_f)N_i^{-1}$, where N_f and N_i are, respectively, the final and initial number of paralarvae in the rearing tank of each diet treatment.

Prior to sample collection, paralarvae were anaesthetized with Cl_2Mg6H_2O 0.4M (Panreac-Química SA, Barcelona, Spain), according to the methodology described by Messenger *et al.* (1985). Afterwards, VML was measured under a magnifying glass (5× magnification, SMZ-10A; Nikon Corporation, Tokyo, Japan), and DW was determined by drying paralarvae samples in an oven for 24 h at 110 °C. The remaining paralarvae of each given tank were also collected and stored at –80 °C for lipid content determinations.

Artemia sp. production

Artemia sp. (EG Sep-Art; INVE Aquaculture, Dendermonde, Belgium) juveniles were reared from nauplii, which were obtained according to the methods described by Sorgeloos (1977). Lyophilized *Tetraselmis chuii* (Easy Algae – Fitoplancton marino S.L., Cádiz, Spain) was used for growing and to improve its nutritional value. A volume of 100 mL containing 4 g of *T. chuii* was daily added to the rearing tanks, which was preceded by 10% of water volume removed from the tank bottom, so rests of phytoplankton and nauplii could be eliminated. *Artemia* sp. was reared for 10 days (reaching the juvenile stage) in 100 L cylinder conical fibreglass tanks, at densities of 10 nauplii mL^{-1} (10^6 per tank) with strong aeration.

Palaemon elegans zoeae production

The *P. elegans* broodstock was captured in intertidal pools at the NE coast of Tenerife (Canary Islands, Spain) during the low tide period. Individuals were reared in cylinder conical fibreglass tanks, with either 500 or 1000 L capacity, in a flow-through seawater system, at a density of 1–2 individuals L^{-1}. Rearing conditions were natural and similar to those described for the octopus broodstock. Shrimps were daily fed *ad libitum* on frozen squid (*L. opalescens*). Oviger females were separated to individual cages (1 cm mesh) in another tank, which allowed the newly born zoeae to swim outside the cage and avoid cannibalism by females. Newly born zoeae were then collected and used as food for octopus.

Grapsus adscensionis zoeae production

The decapod crustacean *G. adscensionis* broodstock was caught at the NE and N coast of Tenerife (Canary Islands, Spain) and was reared in 3000 L cylinder conical fibreglass tanks of a flow-through system. Photoperiod and water conditions were similar also to those described for the octopus broodstock. Tank water column was of ≈10 cm, and water flow was 6 L min^{-1}. Adult crabs were daily fed *ad libitum* on a diet consisting of 50% (w/w) of frozen mackerel (*Scomber scombrus*) and squid (*L. opalescens*). Newly hatched crab zoeae were collected on a 500 μm mesh placed at the end of the flow-water system. After a thorough separation of zoeae from possible algae and other organisms, zoeae were used as food for octopus paralarvae.

Biochemical composition analysis

Total lipids (TL) were extracted, using chloroform:methanol (2 : 1) as extracting solvents and butylhydroxytoluene (BHT) as antioxidant, and gravimetrically determined (Christie 1982). Lipid classes (LC) were determined by high performance thin layer chromatography (HPTLC) and densitometry [CS-9001PC (DUAL-WAVELENGHT FLYING SPOT SCANNER; Shimadzu, Kyoto, Japan)],

following the method described by Olsen & Henderson (1989). Fatty acids (FA) were obtained by acid-catalysed transmethylation with 1% sulphuric acid (v/v) in methanol. The resultant fatty acid methyl esters (FAME) were purified by thin layer chromatography (TLC) (Christie 1982). FAME were separated and quantified using a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (250 °C) and a fused silica capillary column SupelcowaxTM10 (30 m × 0.32 mm I.D.). Helium was used at the carrier gas. Individual FAME were identified by referring to well characterized standards (PUFA n° 3; Supelco Park, Bellefonte, PA, USA). Dry matter (DM) was determined at 110 °C until constant weight was obtained, in agreement with AOAC (2006) which is an adaptation of the Horwirtz method (1980).

BHT, potassium chloride and potassium bicarbonate were supplied by Sigma Chemical Co (St. Louis, MO, USA). TLC (20 cm × 20 cm × 0.25 mm) and HPTLC (10 cm × 10 cm × 0.15 mm) plates, percolated with silica gel (without fluorescent indicator), were purchased from Macheren-Nagel (Düren, Germany). All organic solvents for GC used were of reagent grade and were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (Taufkirchen, Germany) and Panreac-Química SA (Barcelona, Spain).

Data analysis

Results are presented as means ± SD. Data were checked for normal distribution with the one-sample Kolmogorov–Smirnoff test (Zar 1999) as well as for homogeneity of the variances with the Levene test (Zar 1999) and, when necessary, arcsine transformation was performed (Fowler *et al.* 1998).

Growth (DW, VML, IGR), SR, DM, TL, LC and FA contents were assessed by one-way analysis of variance (ANOVA) followed by a Tukey's *post hoc* test (Zar 1999). When normal distribution and/or homogeneity of the variances were not achieved, data were subjected to the Kruskall–Wallis non-parametric test, followed by a Games–Howell non-parametric multiple comparison test (Zar 1999). In all statistical tests used, P values of less than 0.05 were considered statistically different.

Differences between DM, TL, LC and FA composition of 9-day-old paralarvae and corresponding live preys used in its culture were tested using a Student's t-test. The statistical analysis was performed using the SPSS package version 15.0 (SPSS Inc, Chicago, IL, USA).

Results and discussion

Dry weight (DW) and ventral mantle length (VML) of hatchlings and paralarvae, as well as IGR and SR are shown in Table 1. The VML of hatchlings (1.20 ± 0.05 mm) was within the range size reported by Iglesias *et al.* (2007) and Quintana (2009). On the other hand, the dry weight (DW – 0.17 ± 0.02 mg) was lower than that reported in previous studies (Navarro & Villanueva 2000; Iglesias *et al.* 2004, 2013; Quintana 2009; Domingues *et al.* 2013). Nonetheless, the size and weight of the hatchlings depends on many factors, such as female weight, broodstock diet, water temperature, etc. (Quintana 2009), and might not necessarily be indicative of hatchlings quality and survival. The VML of 9-day-old paralarvae was not significantly different from hatchlings or within diet treatments. Nonetheless, differences were observed in DW and IGR within 9-day-old paralarvae ($P < 0.05$). Similar to what was reported by Villanueva (1994), Iglesias *et al.* (2004) and Carrasco *et al.* (2006) the highest DW (0.30 ± 0.03 and 0.27 ± 0.02 mg) and IGR (6.29 ± 1.10 and 5.18 ± 0.96% DW day^{-1}) were attained by paralarvae fed with a diet containing decapod crustacean zoeae (GRA and PAL : ART, respectively; $P < 0.05$; Table 1). Even though, our IGR results were slightly lower than the ones reported by those authors: 8.05, 7.85 and 7.77% for Villanueva (1994), Iglesias *et al.* (2004) and Carrasco *et al.* (2006), respectively.

Similarly, in the present study, the highest SR was attained in GRA (64.83 ± 23.62%) and PAL : ART (55.00 ± 30.50%; Table 1) treatments. Nonetheless, due to the high survival data dispersion observed in all groups, these values were not statistically different from the SR recorded on ART (46.33 ± 25.96%). The present SR was lower to those reported by Seixas *et al.* (2010a) who used enriched *Artemia* sp. juveniles, as sole live prey, and who attained survival rates of 83–95%; to the ones achieved by Villanueva (1995), who fed octopus paralarvae with *Pagurus prideaux* and *Liocarcinus depurator* zoeae and registered a survival rate of 77.2%, both after 10 days of rearing; or even to that attained by Itami *et al.* (1963) who fed the paralarvae with *Palaemon serrifer* zoeae and reached a survival rate of 85.1%. However, our results are similar to those of Seixas *et al.* (2010b) with a SR ranging from 49.3 to 59.4% and even higher than those of Navarro & Villanueva (2000) who reached 11 and 31% both using enriched *Artemia* sp. as prey during 10 days. These results suggest that there is a high variability on paralarvae survival that could not be only dependent upon the type of prey but also of larvae initial condition and/or environmental and zootechnical aspects.

Table 1 Growth and survival of *Octopus vulgaris* 9-day-old paralarvae fed different preys

	Hatchlings	ART	GRA	PAL : ART
DW (mg)	0.17 ± 0.02[a]	0.22 ± 0.03[b]	0.30 ± 0.03[c]	0.27 ± 0.02[c]
VML (mm)	1.20 ± 0.05	1.20 ± 0.13	1.25 ± 0.07	1.20 ± 0.11
IGR (% DW day^{-1})	0.00 ± 0.00	2.79 ± 1.33[a]	6.29 ± 1.10[b]	5.18 ± 0.96[b]
SR (%)	0.00 ± 0.00	46.33 ± 25.96	64.83 ± 23.62	55.00 ± 30.50

DW, dry weight; VML, ventral mantle length; IGR, instantaneous growth rate; SR, survival rate; ART, octopus paralarvae fed with *Artemia* sp.; GRA, octopus paralarvae fed with *Grapsus adcensionis*; PAL : ART, octopus paralarvae fed with cofeeding of *Palaemon elegans* + *Artemia* sp. Results represent means ± SD (n = 15). Values in the same row bearing different superscript letters are significantly different ($P < 0.05$).

On the other hand, this 10-day period may also correspond to the critic rearing point of the exhaustion of the inner yolk reserves, while octopus is feeding on life preys, in a similar fashion to what Sykes *et al*. (2013) described when feeding cuttlefish with frozen food from day 1 after hatching. In spite of these considerations and according to previous and present results, it is evident that crustacean zoeae seems to nutritionally contribute with something else than *Artemia* sp. on behalf of paralarvae rearing performance.

The preys selected for the present study displayed different TL contents, LC (Table 2) and FA profiles (Table 3). *G. adscensionis* displayed the lowest TL content (47.0 ± 4.7 g kg^{-1} of DM; $P < 0.05$) and *P. elegans* the highest one (155.5 ± 56.6 g kg^{-1} of DM; $P < 0.05$; Table 2). Nonetheless, no statistical differences were observed regarding the TL content of *P. elegans* and *Artemia* sp. As previously reported by Viciano *et al*. (2011) and Seixas *et al*. (2010a,b) for paralarvae of 30 and 15 days, respectively, the different TL of preys was not translated into, differences of TL between 9-day-old paralarvae (Table 2). This might indicate that paralarvae are able to preserve its TL content despite the lipid content of preys. In marine finfishes, lipids are the main source of metabolic energy used for growth, reproduction and displacement (Sargent *et al*. 2002). In contrast, adult *O. vulgaris* and cephalopods in general, use protein as main energy source in detriment of a lipid energetic metabolism (Lee 1994; Giménez & Gracía García 2002). Despite, the low digestibility of lipids and the fact that they promote oily faeces when animals are fed on rich lipid diets, they appear as a limiting component for adult octopus growth on its normal diet and egg production (O'Dor *et al*. 1984).

Octopus vulgaris hatchlings TL profile was particularly rich in phosphatidylethanolamine (PE – 32.73 ± 1.08%) and phosphatidylcholine (PC – 13.17 ± 0.63% of TL), within the polar lipid fraction which accounted 64.64 ± 1.64% of TL, and in cholesterol (CHO – 32.27 ± 1.68%; Table 2) within the neutral lipid fraction. Navarro & Villanueva (2000) reported CHO, followed by PC and PE, as the three main LC of paralarvae. Almansa *et al*. (2006) also mentioned that *Sepia officinalis* requires a prey with high levels of PC, PE and CHO. The consistent high contents of those lipid classes in the lipid profiles of *O. vulgaris* hatchlings and other cephalopods may suggest their importance in the development of these species. However, the knowledge about the role of the LC profile in octopus paralarvae development is still scarce and based on data of contents in total phospholipids among the polar lipids and of sterols, free fatty acids, triacylglycerides and esters and waxes, within the neutral ones (Moxica *et al*. 2002; Fuentes *et al*. 2011; Iglesias *et al*. 2013). Only Navarro & Villanueva (2000) presented data of the different polar lipid classes. In the present study, a more complete profile of polar and neutral lipid classes is also given either in octopus paralarvae and live preys. Interestingly, the PE content of hatchlings was 2.5 times higher than the PC and different from the ones of Navarro & Villanueva (2000) who attained 21% of PC and 16% of PE. However, after the rearing period PE contents decreased to 15–17% of total lipids, reaching a value similar to that of PC (between 15.22 and 19.20%) and to those reported by Navarro & Villanueva (2000) in hatchlings and 30-day-old paralarvae (from 12 to 17% of PE and 16–19% of PC). From the experience recorded in our research centre, octopus hatchlings also tend to present similar contents of PC and PE (Reis, D.B., Rodríguez, C., Martín, M.V. & Almansa, E, unpublished data). Interestingly, hatchlings of the present study exhibited a higher content of PE which suffered a strong reduction in 9 days. This could be related with a catabolism of this phospholipid as metabolic source, in the same way to that recorded for the PC in marine fish larvae prior to first feeding (Rainuzzo *et al*. 1992) or even on other phospholipid synthesis (Tocher *et al*. 2008).

It is also noteworthy that the preys that promote higher IGR on octopus paralarvae also presented the highest percentage of neutral lipids (61.09 ± 2.27% in *G. adscensionis* and 60.87 ± 5.11% in *P. elegans* zoeae; $P < 0.05$),

Table 2 Dry matter (g kg^{-1}), total lipid (g kg^{-1} of DM) and lipid class (% of TL) composition of *Octopus vulgaris* hatchlings, live preys and 9-day-old feed paralarvae

		Preys			9-days-old paralarvae		
	Hatchlings	*Artemia* sp.	*Grapsus adscensionis*	*Palaemon elegans*	ART	GRA	PAL : ART
DM	253.1 ± 23.2^{a}	82.2 ± 1.4^{d}	199.4 ± 4.5^{b}	125.7 ± 11.6^{c}	210.5 ± 5.1	252.7 ± 39.3	218.3 ± 7.4
TL	83.1 ± 10.6^{a}	92.8 ± 17.2^{a}	47.0 ± 4.7^{b}	155.5 ± 56.6^{a}	122.6 ± 34.5^{1}	92.8 ± 37.4^{2}	100.2 ± 16.8^{1}
Lipid class							
TPL	64.64 ± 1.64^{aA}	49.62 ± 4.28^{b}	38.91 ± 2.27^{c}	39.13 ± 5.11^{c}	48.16 ± 8.65^{BC1}	43.91 ± 3.15^{B}	54.01 ± 1.69^{C1}
LPC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00^{1}	0.05 ± 0.10^{2}	0.00 ± 0.00^{13}
SM	0.22 ± 0.14	0.50 ± 0.11	0.39 ± 0.22	0.18 ± 0.07	0.31 ± 0.19^{1}	0.43 ± 0.18^{2}	0.42 ± 0.18^{1}
PC	13.17 ± 0.63^{abA}	16.40 ± 2.00^{c}	11.71 ± 0.71^{a}	15.56 ± 1.96^{bc}	15.22 ± 2.64^{ABC1}	16.14 ± 1.38^{B}	19.20 ± 0.19^{C1}
PS + PI	14.38 ± 1.35^{aA}	10.11 ± 1.56^{b}	5.29 ± 0.48^{c}	7.04 ± 1.81^{c}	11.44 ± 2.64^{AB1}	9.32 ± 0.96^{B}	12.48 ± 0.08^{A1}
PG	4.14 ± 1.53	6.32 ± 0.84	4.57 ± 1.02	4.35 ± 1.06	3.86 ± 1.27	2.71 ± 0.50	4.24 ± 0.34^{3}
PE	32.73 ± 1.08^{aA}	16.28 ± 0.40^{b}	16.95 ± 1.56^{b}	11.98 ± 2.24^{c}	17.33 ± 2.39^{B1}	15.26 ± 0.58^{B2}	17.67 ± 2.09^{B1}
TNL	35.36 ± 1.64^{aA}	50.38 ± 4.28^{b}	61.09 ± 2.27^{c}	60.87 ± 5.11^{c}	51.84 ± 8.65^{BC1}	56.09 ± 3.15^{B}	45.99 ± 1.69^{C1}
DAG	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.00 ± 0.37^{b}	2.71 ± 0.56^{b}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CHO	32.27 ± 1.68^{aAB}	24.63 ± 1.18^{b}	16.80 ± 0.61^{c}	14.02 ± 4.20^{bc}	34.33 ± 3.97^{B}	26.30 ± 0.74^{C}	27.13 ± 2.91^{BC1}
FFA	1.32 ± 0.59^{a}	0.00 ± 0.00^{b}	6.76 ± 0.96^{c}	2.05 ± 1.46^{a}	1.30 ± 1.43	2.22 ± 0.95	1.47 ± 0.91^{3}
TAG	0.62 ± 0.18^{aA}	22.85 ± 3.82^{b}	27.53 ± 2.16^{b}	38.04 ± 8.16^{c}	2.83 ± 1.03^{B}	5.86 ± 2.34^{B}	5.81 ± 1.95^{B}
SE	1.16 ± 0.16^{aA}	2.90 ± 2.64^{ab}	8.01 ± 0.67^{c}	4.04 ± 1.09^{bc}	13.38 ± 4.01^{BC}	21.71 ± 1.67^{B}	11.58 ± 0.56^{C}

TL, total lipid; DM, Dry matter; LPC, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; DAG, diacylglycerides; CHO, cholesterol; TAG, triacylglycerides; SE, sterol esters; TPL, total polar lipids; TNL, total neutral lipids; ART, octopus paralarvae fed with *Artemia* sp.; GRA, octopus paralarvae fed with *Grapsus adcensionis*; PAL : ART, octopus paralarvae fed with cofeeding of *Palaemon elegans* + *Artemia* sp. Results represent means ± SD ($n = 4$). Values in the same row bearing different superscript lowercase letters are significantly different among hatchlings and live preys ($P < 0.05$). Values in the same row bearing different superscript uppercase letters are significantly different among hatchlings and 9-days-old feed paralarvae groups ($P < 0.05$).

[1] Similarities to *Artemia* sp. juveniles ($P > 0.05$).

[2] Similarities to *G. adscensionis* zoeae ($P > 0.05$).

[3] Similarities to *P. elegans* zoeae ($P > 0.05$).

and the lowest in CHO (16.80 ± 0.61% in *G. adscensionis* and 14.02 ± 4.20% in *P. elegans* zoeae; Table 2). Navarro & Villanueva (2000, 2003) and Iglesias *et al.* (2007), referred to the need of high proportions of dietary phospholipids and CHO as essential nutritional factors for octopus paralarvae development. In absolute terms, it is possible that a minimum amount in TPL and CHO for paralarvae development was covered in both dietary treatments. On the other hand, it is also possible that the better results obtained in terms of paralarvae IGR were promoted by other factor than zoeae lipid class composition. In any case, this can only be confirmed by performing a doses-answer or lipid metabolism research on LC synthesis or fatty acid esterification into specific LC, without disconsidering the effects of other nutrients in *O. vulgaris* paralarvae development.

After the 9 days of rearing, some similarities within preys and paralarvae LC composition was verified (Table 2). Interestingly, regarding the PAL : ART diet treatment, a higher similarity of paralarvae with *Artemia* sp. juveniles composition was observed. This could be related to a higher ingestion of *Artemia* sp. juveniles by paralarvae or even with the fact that *P. elegans* zoeae could also be predating on the *Artemia* sp. and then being consumed by the paralarvae. According to Bottino *et al.* (1980), shrimp body lipid composition is profoundly affected by diet lipid composition which could, to some extent explain these results. Compared to hatchlings, 9-day-old paralarvae LC composition shifted towards a lower proportion in polar lipids in all groups ($P < 0.05$; Table 2), mostly due to a decrease in PE and an increase in TAG and sterol esters (SE). The CHO levels of 9-day-old paralarvae fed with decapod crustacean zoeae decreased ($P < 0.05$), although in PAL : ART, it was not statistically different from hatchlings. The latter was possibly due to *Artemia* sp. being the prey with highest level of CHO (24.63 ± 1.18% of TL).

Long-chain polyunsaturated fatty acids (LC-PUFA) have been previously suggested as important dietary components for octopus paralarvae development (Navarro & Villanueva 2003). Previous studies (Villanueva 1994; Iglesias *et al.* 2004, 2013; Carrasco *et al.* 2006) were performed on paralarvae feeding, reported that the best rearing results were obtained

Table 3 Fatty acid composition (% of total fatty acids) of *Octopus vulgaris* hatchlings, live preys and 9-day-old paralarvae

		Preys			9-day-old paralarvae		
	Hatchlings	*Artemia* sp.	*Grapsus adscensionis*	*Palaemon elegans*	ART	GRA	PAL : ART
16 : 0	21.05 ± 0.58^{aA}	14.99 ± 0.31^{b}	21.28 ± 0.22^{a}	24.23 ± 3.62^{a}	17.50 ± 1.10^{B}	19.64 ± 0.12^{A}	19.18 ± 1.62^{AB3}
18 : 0	9.40 ± 0.13^{aA}	10.16 ± 0.20^{b}	9.16 ± 0.18^{a}	5.47 ± 0.41^{c}	13.78 ± 0.39^{B}	12.16 ± 0.19^{C}	11.51 ± 0.78^{C}
∑SFA	32.62 ± 0.76^{a}	28.87 ± 1.11^{b}	35.05 ± 0.28^{c}	34.74 ± 2.85^{abc}	33.82 ± 1.54	34.62 ± 0.14^{2}	33.57 ± 2.23^{3}
18:1n-9	2.06 ± 0.04^{aA}	17.18 ± 0.10^{b}	16.80 ± 0.09^{c}	10.81 ± 1.59^{d}	5.96 ± 0.61^{BC}	6.31 ± 0.50^{B}	5.26 ± 0.27^{C}
18:1n-7	1.08 ± 0.04^{aA}	11.29 ± 0.16^{b}	6.44 ± 0.10^{c}	5.78 ± 0.89^{c}	3.63 ± 0.68^{BC}	3.29 ± 0.29^{B}	3.99 ± 0.32^{C}
20:1n-9	4.01 ± 0.05^{aA}	1.18 ± 0.00^{b}	2.55 ± 0.04^{c}	0.93 ± 0.58^{bc}	3.25 ± 0.26^{B}	2.42 ± 0.17^{C2}	2.43 ± 0.33^{C}
∑MUFA	10.67 ± 0.23^{aA}	33.90 ± 0.49^{b}	33.43 ± 0.24^{b}	24.23 ± 2.38^{c}	16.38 ± 0.95^{B}	16.08 ± 0.71^{B}	15.76 ± 1.26^{B}
18:2n-6	0.44 ± 0.01^{aA}	3.17 ± 0.06^{b}	4.41 ± 0.12^{c}	2.99 ± 2.14^{abc}	1.79 ± 0.58^{B}	1.76 ± 0.82^{B}	1.32 ± 0.12^{B3}
20:2n-6	0.00 ± 0.00^{A}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.73 ± 0.07^{B}	1.20 ± 0.09^{C}	0.66 ± 0.05^{B}
20:4n-6	4.56 ± 0.06^{aA}	1.15 ± 0.03^{b}	10.08 ± 0.20^{c}	2.80 ± 1.56^{ab}	4.75 ± 0.54^{A}	12.62 ± 0.86^{C}	6.34 ± 0.57^{B}
22:4n-6	0.00 ± 0.00^{A}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.06^{B}	0.47 ± 0.05^{C}	0.27 ± 0.05^{B}
∑n-6	5.35 ± 0.07^{aA}	4.67 ± 0.09^{b}	15.39 ± 0.34^{c}	6.17 ± 4.05^{abc}	7.63 ± 0.50^{B}	16.78 ± 0.57^{D}	9.04 ± 0.69^{C3}
∑n-6 HUFA	4.78 ± 0.06^{aA}	1.15 ± 0.03^{b}	10.23 ± 0.21^{c}	2.97 ± 1.75^{b}	4.95 ± 0.64^{A}	13.36 ± 0.91^{B}	7.02 ± 0.62^{C}
18:3n-3	0.00 ± 0.00^{aA}	16.10 ± 0.26^{b}	0.81 ± 0.54^{ac}	2.52 ± 1.62^{c}	2.77 ± 0.49^{B}	0.71 ± 0.27^{C2}	1.42 ± 0.18^{D3}
20:3n-3	1.85 ± 0.03^{aA}	0.41 ± 0.01^{b}	0.34 ± 0.01^{c}	0.24 ± 0.17^{bc}	2.01 ± 0.12^{A}	1.38 ± 0.06^{B}	1.52 ± 0.13^{B}
20:5n-3	17.07 ± 0.18^{aA}	9.08 ± 0.30^{b}	9.18 ± 0.09^{b}	15.63 ± 0.72^{a}	17.11 ± 1.45^{A}	12.00 ± 0.26^{B}	16.29 ± 0.73^{A3}
22:6n-3	26.45 ± 0.44^{aA}	0.03 ± 0.06^{c}	2.55 ± 0.06^{b}	13.38 ± 5.42^{a}	15.29 ± 2.02^{B}	14.36 ± 0.59^{B}	17.80 ± 1.01^{C3}
∑n-3	46.72 ± 0.56^{aA}	29.03 ± 0.68^{b}	14.76 ± 0.64^{c}	33.21 ± 4.10^{b}	39.65 ± 2.90^{B}	30.47 ± 0.51^{C}	38.79 ± 0.77^{B3}
∑n-3 HUFA	44.66 ± 0.62^{aA}	9.59 ± 0.34^{b}	12.78 ± 0.03^{c}	29.53 ± 5.90^{d}	33.51 ± 3.71^{B}	27.57 ± 0.62^{C}	35.08 ± 0.63^{B3}
∑PUFA	52.53 ± 0.59^{aA}	33.97 ± 0.75^{b}	30.06 ± 0.76^{c}	38.70 ± 0.73^{d}	46.99 ± 2.78^{B}	47.01 ± 0.86^{B}	47.68 ± 0.61^{B}
Unknown	4.18 ± 0.38^{aA}	3.26 ± 0.90^{ab}	1.74 ± 0.14^{b}	2.33 ± 0.79^{ab}	2.81 ± 0.61^{B1}	2.30 ± 0.30^{B}	2.99 ± 1.63^{AB13}
n-3/n-6	8.74 ± 0.11^{aA}	6.21 ± 0.08^{b}	0.96 ± 0.03^{c}	8.48 ± 6.37^{abc}	5.22 ± 0.57^{B}	1.82 ± 0.08^{D}	4.31 ± 0.37^{C3}
DHA/EPA	1.55 ± 0.02^{aA}	0.00 ± 0.01^{b}	0.28 ± 0.01^{c}	0.85 ± 0.31^{ac}	0.89 ± 0.05^{C}	1.20 ± 0.06^{B}	1.10 ± 0.10^{BC3}
EPA/ARA	3.74 ± 0.03^{aA}	7.89 ± 0.15^{b}	0.91 ± 0.01^{c}	7.39 ± 4.36^{abc}	3.62 ± 0.23^{A}	0.95 ± 0.07^{C2}	2.59 ± 0.33^{B3}
DHA/ARA	5.80 ± 0.10^{aA}	0.03 ± 0.05^{b}	0.25 ± 0.01^{c}	7.25 ± 5.99^{abc}	3.22 ± 0.27^{B}	1.14 ± 0.05^{D}	2.82 ± 0.23^{C3}

Totals include some minor components not shown; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; HUFA, highly unsaturated fatty acids; PUFA, polyunsaturated fatty acids; DHA-22:6n-3; EPA-20:5n-3; ARA-20:4n-6. ART, octopus paralarvae fed with *Artemia* sp.; GRA, octopus paralarvae fed with *Grapsus adcensionis*; PAL : ART- octopus paralarvae fed with cofeeding of *Palaemon elegans* + *Artemia* sp. Results represent means ± SD ($n = 4$). Values in the same row bearing different superscript lowercase letters are significantly different among hatchlings and live preys ($P < 0.05$). Values in the same row bearing different superscript uppercase letters are significantly different among hatchlings and 9-day-old feed paralarvae groups ($P < 0.05$).

[1] Similarities to *Artemia* sp. juveniles ($P > 0.05$).

[2] Similarities to *G. adscensionis* zoeae ($P > 0.05$).

[3] Similarities to *P. elegans* zoeae ($P > 0.05$).

when decapod crustacean zoeae, preys with 35–40% of n-3 highly unsaturated fatty acids (n-3 HUFA; Navarro & Villanueva 2000; Andrés *et al*. 2010), were used. Navarro & Villanueva (2000) also suggested that EPA and DHA, within n-3 HUFA, are the most important FA in octopus paralarvae composition. All selected preys in the present study showed a lower content in PUFA and n-3 HUFA compared with hatchlings (52.53 ± 0.59% and 44.66 ± 0.62% of total FA, respectively), and a different content on DHA (26.45 ± 0.44% of total FA in hatchlings; $P < 0.05$). *P. elegans* zoeae display a lower DHA content of 13.38 ± 5.42%, while *G. adscensionis* zoeae and *Artemia* sp. presented, respectively, 2.55 ± 0.06% and 0.03 ± 0.06% which are minimal values of content for this FA (Table 3). After 9 days of rearing, the DHA content of *O. vulgaris* paralarvae, presented a significant reduction, regardless of the prey provided (Table 3). This reduction in DHA was similar to that verified by Navarro & Villanueva (2000) and Seixas *et al*. (2010a,b). A clear reduction of EPA and DHA during the first days of feeding has also been described in marine fish larvae (Sargent *et al*. 1999, 2002), and despite this initial reduction did not affect larval performance, a diet poor in DHA can potentially damage the neural and visual development, with significant consequences for the larvae physiological and behavioural processes (Sargent *et al*. 1999, 2002).

According to Okumura *et al*. (2005), a DHA/EPA ratio around 1.5 is necessary for paralarvae normal growth and development, although a doses-answer study was not yet performed to corroborate this analysis. In addition, *O. vulgaris* juveniles and adults have a DHA/EPA ratio from 1.1 to 1.7 (Navarro & Villanueva 2003; Miliou *et al*. 2006). In the

present study, hatchlings DHA/EPA ratio was 1.55 ± 0.02, and the highest IGR was accomplished in paralarvae displaying the highest DHA/EPA ratios (1.20 ± 0.06 in GRA and 1.10 ± 0.10 in PAL : ART). Remarkably, Seixas *et al.* (2010a) was able to reach around 20% survival, after 35 days, with a DHA/EPA ratio of only 0.3, suggesting that an increase in the DHA content of *Artemia* sp. did not improve paralarvae growth or survival.

In a general way, the EPA contents of 9-day-old paralarvae were maintained in similar levels to those observed in hatchlings; exception made for paralarvae fed with *G. adscensionis* zoeae (GRA treatment) which displayed a significant reduction in this FA (Table 3). Interestingly, the diet that leaded to a lower content in EPA was the same that promoted a higher content of ARA. An inverse relation between this two FA incorporation was also reported in previous studies (Furuita *et al.* 2003 Miliou *et al.* 2006; Atalah *et al.* 2011) and may indicate a possible competition between these two FA for acylases and transacylases in FA esterification (Sargent *et al.* 1999). In the present study, the preys that provided the highest IGR (decapod crustacean species) were the ones inducing a higher content of ARA and, respectively, the lowest EPA/ARA ratio in paralarvae ($P < 0.05$; Table 3). However, the ideal EPA/ARA ratio for paralarvae normal development is difficult to determine, as this ratio is usually unconsidered and also due to the inconsistent results obtained, in terms of growth and survival, during paralarvae rearing (Navarro & Villanueva 2000, 2003; Okumura *et al.* 2005; Seixas *et al.* 2010a,b; Viciano *et al.* 2011; Iglesias *et al.* 2013). Feeding paralarvae with decapod crustacean zoeae for 15 days, Iglesias *et al.* (2013) attained a similar EPA/ARA ratio than hatchlings (in between 2 and 2.5), for the different experiments. In any case, the zoeae used in that study presented an equivalent content of EPA (19.37%) and ARA (6.58%) comparing with the hatchlings (16.16% of EPA and 7.84% of ARA). On the other hand, Seixas *et al.* (2010a), using different types of enriched *Artemia* sp., obtained an EPA/ARA ratio ranging from 4.2 to 5.9 after 15 days of rearing, while hatchlings presented an EPA/ARA ratio of 2.0, similar to those of Iglesias *et al.* (2013). To determine the ideal EPA and ARA levels for a paralarvae diet or in which way a raise in ARA content could possibly be detrimental for an EPA raise, a doses-answer study would be necessary having in account not only these FA but also DHA as an essential fatty acid.

The importance of ARA in *O. vulgaris* development was previously suggested (Miliou *et al.* 2006; García-Garrido *et al.* 2010; Estefanell *et al.* 2011; Monroig *et al.* 2012a,b) and may be related to the fact that ARA is a substrate for the biosynthesis of eicosanoids, which in this species are known to be responsible for the control of the cardiac function (Agnisola *et al.* 1994). Moreover, adult octopus brain, gonad, skin and gills present high contents of ARA (Monroig *et al.* 2012b). It is also known that ARA is involved in osmoregulation, camouflage and other important functions in marine animals (Bell *et al.* 1995; Bell & Sargent 2003). Moreover, it is likely that at specific stages in the life cycle of cephalopods as in fish, higher levels of ARA may be required to cope with periods of environmental stress (Bell & Sargent 2003).

A decrease on the PUFA and a raise of MUFA content of paralarvae was detected after the rearing period (Table 3). The polar and neutral lipid fractions of *O. vulgaris* paralarvae show a distinct fatty acid profile, with PUFA being preferably esterified into phospholipids and MUFA into the neutral lipids (Viciano *et al.* 2011). In the present study, it was also observed an increase of the neutral lipids and a reduction of the polar lipids fractions on 9-day-old paralarvae. Therefore, the changes into the FA profile might not only be related to prey FA profile, but also with the changes on the lipid classes content. Furthermore, DHA is mainly found in PC and PE of paralarvae (Quintana 2009), and so it is possible that the decrease of DHA content might be related with the decreased of PE.

It is also interesting to point out that neither hatchlings nor preys showed 20:2n-6 or 22:4n-6 in their composition (Table 3). However, these FA were detected in paralarvae. This could indicate the capacity of *O. vulgaris* paralarvae to synthesize these FA, possibly through their precursors 18:2n-6 and 20:4n-6, respectively. In fact, Monroig *et al.* (2012a) recently described this pathway through the action of an elongase of very long-chain fatty acids (Elovl), applying a molecular approach. The present results reinforce those findings. It cannot be overlooked that Monroig *et al.* (2012a) also verified higher elongation efficiency towards n-6 PUFA rather than the n-3. This also reinforces the suggestion made that ARA may play an important role in paralarvae development. *O. vulgaris* tissues possess an enzyme with Δ5-desaturation activity that participates in the endogenous production of EPA and ARA from 20:4n-3 and 20:3n-6, respectively (Monroig *et al.* 2012b). Despite that, preys contents on these precursors are normally low, (Navarro & Villanueva 2000; Seixas *et al.* 2008, 2010b) as also recorded in this study, and for this reason, Monroig *et al.* (2012b), defined ARA and EPA as essential fatty acids (EFA), contradicting the finding of Miliou *et al.* (2006) who defined ARA as a non-essential FA for this species.

According to Lee (1994), a diet rich in nutrients is useless if the nutrients (such as proteins, lipids, carbohydrates, vitamins) are not adequately assimilated and utilized. To promote the latter, the interactions and competitions between FA biosynthesis must be considered, as well as the role exerted by other nutrients present in this diet, such as specific lipid classes or aminoacids, oligoelements, carotenoids and vitamins. Clearly, there is a need to understand what substrates are used for energy and for growth, and if these necessities change according to temperature/geographical location. In addition, other culture variables such as a possible higher number of preys ingested should not be overlooked.

Using a short trial duration of 9 days, it was possible to obtain enough octopus samples for biochemical analysis and to detect an effect of preys on polar and neutral lipid classes, fatty acid composition and growth at early stages of octopus paralarvae. Some similarities between paralarvae and its prey lipid composition, mainly on lipid class profiles, were also determined. The highest IGR achieved when decapod crustacean zoeae were supplied, possibly indicates that zoeae might nutritionally contribute with something else than *Artemia* sp. to paralarvae rearing performance which may have some relation to the DHA/EPA ratio and/or ARA levels. Interestingly, the diet that leaded to a higher content of ARA was the same that promoted a lower content of EPA. For this reason, a dietary balance EPA/ARA ratio might be significant in this species nutrition without disconsidering DHA as an essential fatty acid. Additionally, the changes observed in the FA profile might not only be related to prey FA profile, but also to the metabolism of specific lipid classes.

Acknowledgements

This work was partially supported by the research projects PRESAPUL (2009–2011), cod. PI SolsubC200801000162, funded by the Canarian Government (ACIISI), and OCTOPHYS (AGL2010-22120-C03-01-MICINN), funded by the Spanish Government. A.V. Sykes (SFRH/BPD/36100/2007) and D.B. Reis (SFRH/BD/76863/2011) wish to thank Fundação para a Ciência e a Tecnologia for their grants.

References

Agnisola, C., Venzi, R., Mustafa, T. & Tota, B. (1994) The systemic heart of *Octopus vulgaris*: effects of exogenous arachidonic acid and capability for arachidonate metabolism. *Mar. Biol.*, **120**, 47–53.

Almansa, E., Domingues, P., Sykes, A., Tejera, N., Lorenzo, A. & Andrade, J.P. (2006) The effects of feeding with shrimp or fish fry on growth and mantle lipid composition of juvenile and adult cuttlefish (*Sepia officinalis*). *Aquaculture*, **256**, 403–413.

Andrés, M., Estévez, A., Simeó, C.G. & Rotllant, G. (2010) Annual variation in the biochemical composition of newly hatched larvae of *Maja brachydactyla* in captivity. *Aquaculture*, **310**, 99–105.

Atalah, E., Hernández-Cruz, C.M., Benítez-Santana, T., Ganga, R., Roo, J. & Izquierdo, M. (2011) Importance of the relative levels of dietary arachidonic acid and eicosapentaenoic acid for culture performance of gilthead seabream (*Sparus aurata*) larvae. *Aquacult. Res.*, **42**, 1279–1288.

Bell, J.C. & Sargent, J.R. (2003) Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture*, **218**, 491–499.

Bell, J.C., Castell, J.D., Tocher, D.R., MacDonald, F.M. & Sargent, J.R. (1995) Effects of different dietary arachidonic:docosahexaenoic acid ratios on phospholipid fatty acid composition and prostaglandin production in juvenile turbot (*Scophthalmus maximus*). *Fish Physiol. Biochem.*, **14**, 139–151.

Bottino, N.R., Gennity, J., Lilly, M.L., Simmons, E. & Finne, G. (1980) Seasonal and nutritional effects on the fatty acids of three species of shrimp, *Penaeus seriferus*, *P. aztecus* and *P. duorarum*. *Aquaculture*, **19**, 139–148.

Carrasco, J.F., Arronte, J.C. & Rodríguez, C. (2006) Paralarval rearing of the common octopus, *Octopus vulgaris* (Cuvier). *Aquac. Res.*, **37**, 1601–1605.

Christie, W.W. (1982) Lipid Analysis, 2nd edn, pp. 207. Pergamon Press, Oxford.

Domingues, P., Sánchez, F.J., Iglesias, J., Otero, J.J., Pazos, G. & Linares, F. (2013) Variations in weight and proximate composition of newly hatched *Octopus vulgaris* (Cuvier 1797) paralarvae. *J. Appl. Aquacult.*, **25**, 219–226.

Estefanell, J., Socorro, J., Tuya, F., Izquierdo, M. & Roo, J. (2011) Growth, protein retention and biochemical composition in *Octopus vulgaris* fed on different diets based on crustaceans and aquaculture by-products. *Aquaculture*, **322–323**, 91–98.

Fowler, J., Cohen, L. & Jarvis, P. (1998) Practical Statistics for Field Biology, 2nd edn, pp. 259. Wiley, West Sussex.

Fuentes, L., Sánchez, F.J., Lago, M.J., Iglesias, J., Pazos, G. & Linares, F. (2011) Growth and survival of *Octopus vulgaris* (Cuvier 1797) paralarvae fed on three *Artemia* -based diets complemented with frozen fish flakes, crusher zooplankton and marine microalgae. *Sci. Mar.*, **75**, 771–777.

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

García-Garrido, S., Hachero-Cruzado, I., Garrido, D., Rosas, C. & Domingues, P. (2010) Lipid composition of the mantle and digestive gland of *Octopus vulgaris* juveniles (Cuvier, 1797) exposed to prolonged starvation. *Aquacult. Int.*, **18**, 1223–1241.

Giménez, F.A. & Gracía García, B. (2002) Growth and food intake models in *Octopus vulgaris* Cuvier (1797): influence of body weight, temperature, sex and diet. *Aquacult. Int.*, **10**, 361–377.

Hanlon, R.T. & Messenger, J.B. (1996) Cephalopod Behaviour, pp. 232. Cambridge University Press, Cambridge.

Horwitz, W. (1980) AOAC official method 950.46 In: AOAC (2006) Official Methods of Analysis of AOAC Internationall (Horwitz W. & Latimer Jr, G.W. assistant eds). 18th edn, pp. 1018. AOAC International, Gaithersburg, MD, USA.

Iglesias, J., Otero, J.J., Moxica, C., Fuentes, L. & Sánchez, F.J. (2004) The completed life cycle of the octopus (*Octopus vulgaris*, Cuvier) under culture conditions: paralarval rearing using

Artemia and zoeae, and first data on juvenile growth up to 8 months of age. *Aquacult. Int.*, **12**, 481–487.

Iglesias, J., Sánchez, F.J., Bersano, J.G.F. *et al.* (2007) Rearing of *Octopus vulgaris* paralarvae: present status, bottlenecks and trends. *Aquaculture*, **266**, 1–15.

Iglesias, J., 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. *Aquacult. Int.*, **22**, 1041–1051.

Itami, K., Izawa, Y., Maeda, S. & Nakay, K. (1963) Notes on the laboratory culture of the octopus culture. *Bull. Jpn. Soc. Sci. Fish.*, **29**, 514–520.

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

Mangold, K. (1983) Food, feeding and growth in cephalopods. *Mem. Nat. Mus. Victoria*, **44**, 81–93.

Messenger, J.B., Nixon, M. & Ryan, K.P. (1985) Magnesium-chloride as an anesthetic for cephalopods. *Comp. Biochem. Phys. C*, **82**, 203–205.

Miliou, H., Fintikaki, M., Tzitzinakis, M., Kountouris, T. & Verriopoulos, G. (2006) Fatty acid composition of common octopus, *Octopus vulgaris*, in relation to rearing temperature and body weight. *Aquaculture*, **256**, 311–322.

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 characterization and tissue distribution of a fatty acyl elongase. *Aquaculture*, **360–361**, 45–53.

Monroig, O., Navarro, J.C., Dick, R., Alemany, F. & Tocher, D.R. (2012b) Identification of a Δ5-like fatty acyl desaturase from the cephalopod *Octopus vulgaris* (Cuvier 1797) involved in the biosynthesis of essential fatty acids. *Mar. Biotechnol.*, **14**, 411–422.

Moxica, C., Linares, F., Otero, J.J., Iglesias, J. & Sánchez, F.J. (2002) Cultivo intensivo de paralarvas de pulpo, *Octopus vulgaris* Cuvier, 1797, en tanques de 9 m^3. *Bol. Inst. Esp. Oceanogr.*, **18**, 31–36.

Navarro, J. & 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. & 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.

O'Dor, R.K., Mangold, K., Boucher-Rodoni, R., Wells, M.J. & Wells, J. (1984) Nutrient absorption, storage and remobilization in *Octopus vulgaris*. *Mar. Behav. Physiol.*, **11**, 239–258.

Okumura, S., Kurihara, A., Iwamoto, A. & Takeuchi, T. (2005) Improved survival and growth in *Octopus vulgaris* paralarvae by feeding large type Artemia and Pacific sandeel, *Ammodytes personatus*. *Aquaculture*, **244**, 147–157.

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.

Quintana, D. (2009) Valoración de los requerimientos nutricionales de reproductores de pulpo común (*Octopus vulgaris*). Tesis de Doctorado, Universidad de La Laguna.

Rainuzzo, J.R., Reitan, K.I. & Jørgensen, L. (1992) Comparative study on the fatty acids an lipid composition of four marine fish larvae. *Comp. Biochem. Phys. B*, **103**, 21–26.

Roura, A., González, A.F., Redd, K. & Guerra, A. (2012) Molecular prey indentification in wild *Octopus vulgaris* paralarvae. *Mar. Biol.*, **159**, 1335–1345.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J. & Tocher, D. (1999) Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture*, **179**, 217–229.

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002) The lipids. In: Fish Nutrition, 3rd edn (Halver, J.E. & Hardy, R.W. eds), pp. 181–257. Academic Press Inc., San Diego, CA.

Scherbakova, A., Riera, R., Almansa, E., Felipe, B., Rodríguez, C., Reis, D., Andrade, J.P. & Sykes, A.V. (2011) Reproductive investment and fecundity of the red rock crab (*Grapsus adscensionis*) in Tenerife (Canary Islands, Atlantic Ocean) (Decapod, Grapsidae). *Vieraea*, **39**, 139–147.

Seixas, P., Rey-Méndez, M., Valente, L.M.P. & Otero, A. (2008) Producing *Artemia* as prey for *Octopus vulgaris* paralarvae with different microalgal species of controlled biochemical composition. *Aquaculture*, **283**, 83–91.

Seixas, P., Rey-Méndez, M., Valente, L.M.P. & Otero, A. (2010a) High DHA content in *Artemia* is ineffective to improve *Octopus vulgaris* paralarvae rearing. *Aquaculture*, **300**, 156–162.

Seixas, P., Otero, A., Valente, L.M.P., Dias, J. & Rey-Méndez, M. (2010b) Growth and fatty acid composition of *Octopus vulgaris* paralarvae fed with enriched *Artemia* or co-fed with inert diet. *Aquacult. Int.*, **18**, 1121–1135.

Sorgeloos, P. (1977) Decapsulation of *Artemia* cysts: a simple technique for the improvement of the use of brine shrimp in aquaculture. *Aquaculture*, **12**, 311–315.

Sykes, A.V., Domingues, P.M., Correia, M. & Andrade, J.P. (2006) Cuttlefish culture – state of the art and future trends. *Vie Milieu*, **56**, 129–137.

Sykes, A.V., Gonçalves, R. & Andrade, J.P. (2013) Early weaning of cuttlefish (*Sepia officinalis*, L.) with frozen grass shrimp (*Palaemonetes varians*) from the first day after hatching. *Aquacult. Res.*, **44**, 1815–1823.

Tocher, D.R., Bendiksen, E.A., Campbell, P.J. & Bell, J.G. (2008) The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture*, **2058**, 21–34.

Vaz-Pires, P., Seixas, P. & Barbosa, A. (2004) Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. *Aquaculture*, **238**, 221–238.

Viciano, E., Iglesias, J., Lago, M.J., Sánchez, F.J., Otero, J.J. & Navarro, J.C. (2011) Fatty acid composition of polar and neutral lipid fractions of *Octopus vulgaris* Cuvier, 1797 paralarvae reared with enriched on-grown *Artemia*. *Aquacult. Res.*, **42**, 704–709.

Villanueva, R. (1994) Decapod crab zoeae as food for rearing cephalopod paralarvae. *Aquaculture*, **128**, 143–152.

Villanueva, R. (1995) Experimental rearing and growth of planktonic *Octopus vulgaris* from hatching to settlement. *Can. J. Fish. Aquat. Sci.*, **52**, 2639–2650.

Villanueva, R. & Norman, M.D. (2008) Biology of the planktonic stages of benthic octopuses. *Oceanogr. Mar. Biol. Annu. Rev.*, **46**, 105–202.

Zar, J.H. (1999) Biostatistical Analysis, pp. 619. Prentice-Hall, Englewood Cliffs, NJ.