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Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements

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Abstract

We report here the main lipid classes and the fatty acid composition from the total lipids of hatchlings of three cephalopod species: *Sepia officinalis*, *Loligo vulgaris* and *Octopus vulgaris*, as well as the lipid composition of two selected crustaceans that have been used previously with success as food resource for rearing cephalopod hatchlings: zoeae of *Pagurus prideaux* and the mysidacean *Acanthomysis longicornis*. Additionally, we report the lipid class and fatty acid composition of two cultures of *O. vulgaris* paralarvae reared with enriched *Artemia* juveniles, and with enriched *Artemia* juveniles plus a prepared pelleted diet. From their lipid composition and that of their natural food, it can be deduced that cephalopod paralarvae and juveniles must require a food rich in polyunsaturated fatty acids (PUFA), phospholipids and cholesterol and with a moderate content in neutral lipids. There was a clear influence of the lipid composition of the food on the lipids of cultured octopus paralarvae both at the level of lipid class and fatty acid composition. The cultured octopus paralarvae showed a lower content of PUFA as compared with the newly hatched individuals. Co-feeding techniques based on the use of polar lipid and PUFA enriched *Artemia* together with palatable pellets seemed to be a possible way to improve paralarval and juvenile cephalopod culture beyond the experimental scale.

Keywords: Lipids; Fatty acids; Cephalopods; Octopus; Squid; Cuttlefish

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1. Introduction

Cephalopods have been cultured through the complete life cycle and multiple generations only at an experimental scale or, in few numbers for biomedical research. The cuttlefish *Sepia officinalis* (Pascual, 1978; Boletzky, 1983; Forsythe et al., 1994; Hanley et al., 1998) and *Sepioteuthis lessoniana* (Lee et al., 1994) seem to be the most easily cultured due, among other reasons, to the large size of the hatchlings. Many cephalopod species, mostly squids and octopods, have a planktonic posthatching stage, and the term paralarva has been proposed for these stages (Young and Harman, 1988). In other cephalopods, such as the cuttlefishes, hatchlings are large and immediately benthic like the adults and thus are referred to as juveniles. Paralarvae and juveniles are active swimmers and rapid growing forms that require substantial amounts of food (Vecchione, 1991; Villanueva and Nozais, 1996; Villanueva et al., 1995). In fact, subadult and adult cephalopods show high metabolic rates and fast growth which are sustained by high feeding rates (O'Dor and Wells, 1987). Digestive capability of paralarval stages (Boucaud-Camou and Roper, 1995, 1998) indicate a carnivorous diet as in juveniles (Boucaud-Camou et al., 1985) and adults (Boucher-Rodoni et al., 1987).

The culture of cephalopods is becoming an important area of interest due to their rapid growth ($>5\%$ body weight day^{-1}) (Forsythe and Van Heukelem, 1987) and high market price. A suitable species is the common octopus *Octopus vulgaris*, due to its rapid growth and high food conversion (Mangold and Boletzky, 1973; Mangold, 1983). At present, the commercial culture of *O. vulgaris* in Spanish waters is limited to ongrowing subadults captured from the wild, to adult sizes (Iglesias et al., 1997; Rama-Villar et al., 1997). Octopus with planktonic stages like *O. vulgaris*, have been successfully reared through their paralarval stages only at an experimental scale (Itami et al., 1963; Villanueva, 1995). The paralarval culture of octopus and early stages of most cephalopod species, still being a bottleneck. The suitable food for rearing these early stages has been an unresolved problem. At present, most of the live prey used for successfully rearing early stages are collected from the sea, as zooplankton for paralarval squids (Yang et al., 1986; Hanlon et al., 1979, 1989) or mysids and palaemonids for juvenile cuttlefishes and squids (Forsythe et al., 1994; Lee, 1994), but this method requires a substantial input of time and effort. On the other hand, laboratory hatched decapod zoeae have been used as food for paralarval octopods and squids (Itami et al., 1963; Villanueva, 1994, 1995), but the use of this food item requires a risky cumbersome parallel culture of crustaceans that seems impractical beyond the experimental scale systems.

Aside from the problems related to food size and quantity, there seem to be other problems associated with food quality. Since cephalopods have a predominant amino acid metabolism (Lee, 1994), research on the lipid and fatty acid requirements have been somewhat neglected to the point that these are scarcely known at present. Adult cephalopods are rich in long chain polyunsaturated fatty acids (PUFA) (Jangaard and Ackman, 1965; Nash et al., 1978), their oil, being a good source of these compounds often used in aquaculture to supplement feeds (Southgate and Lou, 1995; Knauer and Southgate, 1997). As far as we are aware, there are no reports on the fatty acid composition of paralarval and juvenile cephalopods. According to the fatty acid compo-

sition of the adults, and to the generic composition of their natural food, the paralarvae must require high amounts of these fatty acids. This hypothesis is further substantiated by the fact that earlier attempts to culture octopus and squid paralarvae with the brine shrimp *Artemia* failed (Boletzky and Hanlon, 1983; Hamazaki et al., 1991). It is known that *Artemia*, in general, lacks long chain PUFA essential for marine animals, like eicosapentaenoic acid (EPA, 20:5$n-3$) which may be present in low amounts or absent in the lipid of the brine shrimp, and docosahexaenoic acid (DHA, 22:6$n-3$) which is basically absent (Navarro et al., 1992, 1993a).

The aim of the present work was to take a first insight on the nutritional requirements of the paralarval and juvenile stages of cephalopods in culture. First, we determined the main lipid classes and the fatty acid composition of the newly hatched individuals of three cephalopod species that represent the three main cephalopod orders, all of them of high commercial interest: the cuttlefish *S. officinalis*, the European squid *L. vulgaris* and the common octopus *O. vulgaris*. Second, we analysed the same lipid composition of two selected crustaceans that have been successfully used previously as food resources for rearing cephalopod hatchlings, under experimental cultures: zoeae of the hermit crab *Pagurus prideaux* and the mysidacean shrimp *Acanthomysis longicornis* (Villanueva, 1994). Finally, we report the lipid class and fatty acid composition of two cultures of *O. vulgaris* paralarvae reared with enriched *Artemia* juveniles, and with enriched *Artemia* juveniles plus a prepared pelleted diet.

2. Materials and methods

2.1. Collection of material

Cephalopod eggs were collected off Vilanova i la Geltrú, Barcelona (NW Mediterranean) by means of the local ceramic pot fishery for octopus. The pot line was placed near the coast, between 10 and 25 m depth. Egg masses of *S. officinalis* and egg capsules of *L. vulgaris*, attached to the fishery line were collected on board and transported to the lab the same day (February 1998). Eggs were incubated at ambient temperature (mean: 15.2°C, range: 14.7–15.6°C), using an open-circuit of filtered sea water at the ICM, Barcelona. During February and March 1998, active, healthy individuals of the three cephalopod species: *S. officinalis*, *L. vulgaris* and *O. vulgaris*, recently hatched in aquaria were collected for analysis.

Additionally, two crustacean species were analyzed for lipid and fatty acid composition. Ovigerous females of the hermit crab *P. prideaux* were collected from the by-catch of the craft fishery from the same place and depth as above, in January 1998. Ovigerous females were transported to the lab, maintained as described in Villanueva (1994) and the recent hatched zoeae used for analysis. Mysids (*A. longicornis*) were collected in May 1998, off Badalona, Barcelona, by a hand net, at 10 m depth using scuba diving. The same day the individuals were preserved for analysis.

Freshly hatched individuals of cephalopods and crustaceans were collected and preserved during the first 24 h after hatching in aquaria. The samples were collected using a hand net, washed in freshwater, then placed in blotting paper to remove the

water, and weighed using an Ohaus Analytical Plus AP250D-O microbalance. Samples were preserved in chloroform/methanol (2:1, v/v) with 0.01% butylated hydroxytoluene (w/v) as antioxidant. From each sample three aliquot replicates were used for dry weight determinations after drying in an oven for 48 h at 100°C.

2.2. Rearing experiments of *O. vulgaris* paralarvae

O. vulgaris broodstock collected from local craft fishery was maintained using the open-circuit seawater system of the ICM. Females (1–2 kg) were placed with males of similar size during 2–3 days to ensure mating. Fertilised females were isolated and maintained in 200-l individual cylindrical plastic tanks. An overabundance of food was supplied using frozen crabs (*Carcinus maenas*) and sardine (*Sardina pilchardus*). All fertilised females were maintained in the dark with the aim of accelerating their sexual maturation (Wells, 1978; Zúñiga et al., 1995). Some of them were also kept at warm temperature by heating the water to 19–21°C with the same purpose (Villanueva, 1995). Embryonic development of the egg masses and hatching time were controlled according to the rearing needs by means of the temperature of the water, since this is the main factor that modulates the duration of the embryonic period (Boletzky, 1989).

A 700-l semi-closed seawater system connected to the open-circuit of the ICM was used for the paralarval rearing experiments. Seawater was filtered through 0.2 μm and daily renovation rates were 10–40%. The semi-closed system was equipped with a biological filter, protein skimmer, UV lamps, ozonizer and temperature control. Two rearing experiments of *O. vulgaris* from hatching to 30 days were carried out. *Artemia* biomass and pellets were used as food.

Artemia (AF, Artemia Systems) nauplii were grown in seawater at 25–30°C under constant illumination during 7–10 days using 45-l cylindro-conical plastic tanks. *Artemia* cultures were fed *Dunaliella viridis* algae to obtain *Artemia* biomass composed of 1–3 mm total length individuals. This prey size was selected because it had been previously used with success for rearing *O. vulgaris* paralarvae (Villanueva, 1994, 1995). The *Artemia* biomass was enriched in seawater for 24 h at 28°C with one of the following enrichment diets: (a) Diet SS: DC Super Selco (Artemia Systems) 0.6 g l^{-1} and, (b) Diet HB: DC Super Selco 0.6 g l^{-1}, cod liver oil (Acofarma Laboratories) 0.6 g l^{-1}, vitamin C (Sigma Products) 0.3 g l^{-1}, Hipramin-B (Hipra Laboratories) 0.4 ml l^{-1}. Fifty thousand IU l^{-1} of sodium G-penicillin and 50 mg l^{-1} streptomycin sulphate (Sigma) were added to both enrichment media to retard bacterial growth.

The composition and formulation of the pelleted diet in dry weight (%) was: frozen euphausiids (MBF) 30%, squid (*Todarodes sagittatus*) powder (Rieber and Son) 18%, fish hydrolysate (Divaq) 18%, DC Super Selco 8%, phosphatidylcholine (PC, Sigma) 5%, cholesterol (Sigma) 3%, vitamin complex (Kurios) 5%, mineral complex (Warner Lambert) 5%, gelatine (Royal) 7.975%, ethoxyquin (Sigma) 0.025%. Ingredients were mixed with an electric blender, pelletized and dried at room temperature during 48 h. The percentage of moisture was 6%. The pellets were sieved to obtain particle size of 250–500 μm and were stored at −20°C before use. The colour of the pellet was reddish brown.

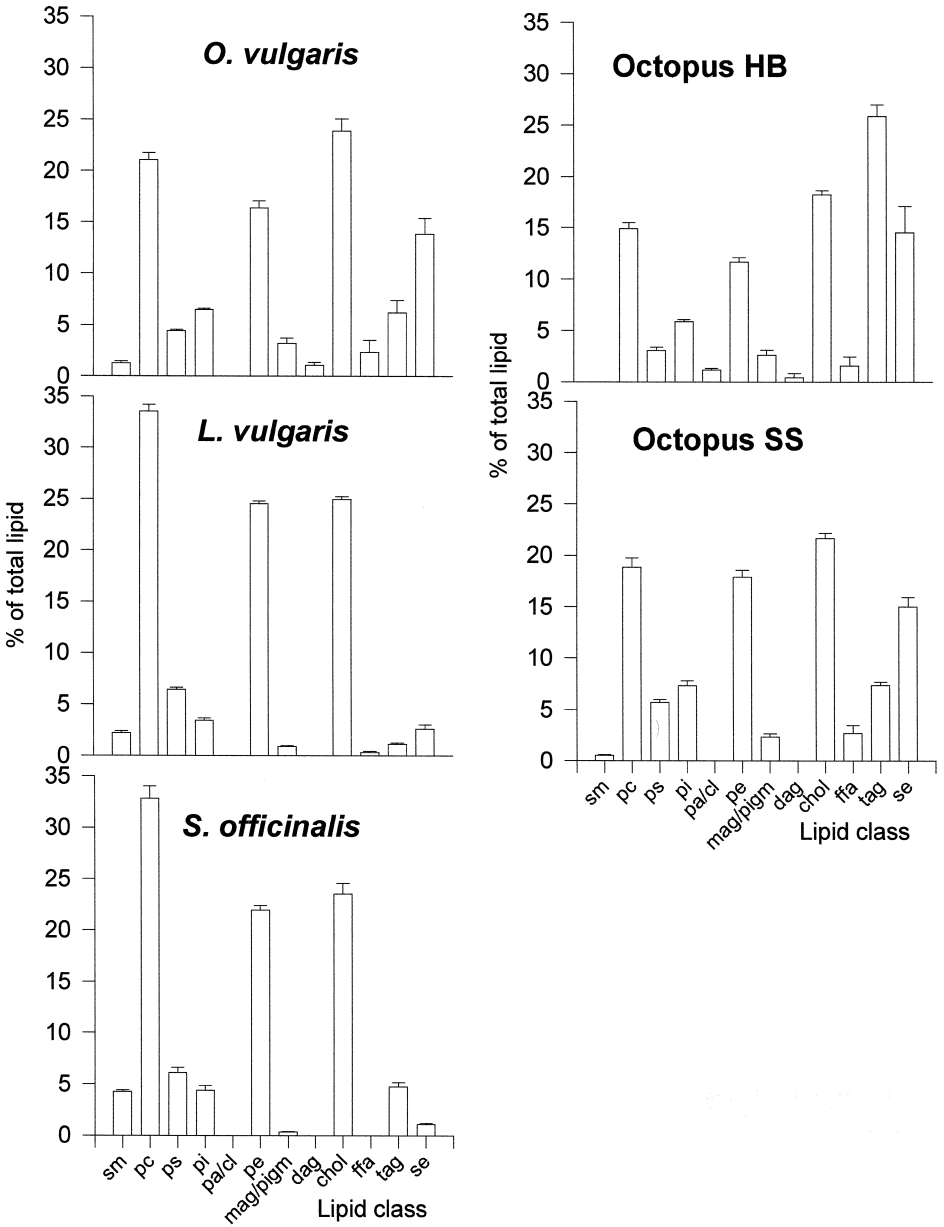

Fig. 1. Lipid class composition (% of the total lipid) of early stages of cephalopods: lpc: lysophosphatidylcholine, sm: shingomyelin, pc: phosphatidylcholine, ps: phosphatidylserine, pi: phosphatidylinositol, pa/cl: phosphatidic acid/cardiolipin, pe: phosphatidylethanolamine, pigm: pigments, mag/dag: mono/di acylglycerides, chol: cholesterol, tag: triacylglycerides, ffa: free fatty acids, se: sterol esters. Data are the mean of four replicates. Error bars are the standard deviation.

Table 1
Total lipid and total fatty acids (% of dry weight), and fatty acids from total lipids (wt.%) of early stages of cephalopods
Data are the means of three replicates. Standard deviations were below 10%.
0.0 are values below 0.09.

Fatty acid	Hatchlings			Paralarval culture	
	O. vulgaris	*S. officinalis*	*L. vulgaris*	Octopus HB	Octopus SS
< 14:0	1.0	0.2	0.4	1.6	0.8
14:0	1.8	2.7	2.1	0.6	0.5
14:1				0.2	0.2
15:0	0.2	0.2	0.5	0.2	0.2
15:1					0.1
16:0	17.5	22.3	22.1	12.1	15.7
16:1$n-9$	0.7		0.1	0.3	0.2
16:1$n-7$	0.5	0.2	0.2	1.5	0.7
16:1$n-5$	0.4				
16:2		0.1		0.2	0.2
17:0	1.4	1.0	1.7	1.4	1.2
16:3	0.1	0.1	0.2	0.3	0.3
16:4		0.1	0.7		0.1
18:0	6.3	8.7	9.1	11.4	12.2
18:1$n-11$	1.4	0.4	0.5		0.7
18:1$n-9$	4.0	4.0	2.8	11.7	5.3
18:1$n-7$	1.6	1.1	1.3	6.7	3.9
18:1$n-5$				0.2	
18:2$n-6$	3.3	0.4	0.3	2.9	1.9
18:3$n-6$					0.1
18:3$n-3$				2.1	1.0
18:4$n-3$					0.1
20:0	0.1	0.2	0.3	0.3	0.2
20:1$n-9$	4.6	5.4	3.5	4.0	2.7
20:1$n-7$			0.4	0.3	0.3
20:2$n-6$	0.9	0.5	0.4	1.1	0.9
20:3$n-6$					0.2
20:4$n-6$	7.3	0.9	3.3	4.9	7.3
20:3$n-3$	0.7	0.4	0.6	1.2	1.2
20:4$n-3$				0.3	0.1
20:5$n-3$	12.6	14.3	14.5	17.4	14.4
22:0					0.1
22:1$n-11$	0.6	0.1	0.1	0.4	0.3
22:1$n-9$				0.5	0.4
22:2$n-6$			0.1		0.1
22:4$n-6$	0.9		0.2		0.2
22:5$n-6$	0.5	0.5	0.6	0.2	0.3
22:5$n-3$	1.7	0.5	1.1	1.5	1.0
22:6$n-3$	21.2	32.8	29.3	9.8	19.1
Total	90.9	96.6	96.2	95.0	94.3
Saturated	27.2	35.0	35.7	25.9	30.1
Monoenes	13.5	11.1	9.0	25.6	14.8
Polyunsaturated	49.2	50.3	51.1	41.9	48.6
$n-3$	36.2	48.0	45.5	32.5	37.0
$n-6$	12.9	2.1	4.7	8.9	11.1

Table 1 (continued)

Fatty acid	Hatchlings			Paralarval culture	
	O. vulgaris	*S. officinalis*	*L. vulgaris*	Octopus HB	Octopus SS
PUFA $n-3$	36.2	48.0	45.5	30.2	35.9
PUFA $n-6$	9.6	1.7	4.5	6.0	9.1
$n-3/n-6$	2.9	23.4	9.6	3.6	3.3
dha/epa	1.7	2.3	2.0	0.6	1.3
% total fatty acids	4.6	4.6	4.1	7.2	5.4
% total lipid	13.4	12.5	10.8	25.1	13.7

2.2.1. *O. vulgaris paralarval culture HB*

The rearing experiment started with a total of 1250 freshly hatched *O. vulgaris* paralarvae divided in two cylindric polyethylene plastic tanks. The characteristics of these tanks and general rearing conditions have been described elsewhere (Villanueva, 1995). Tank volume was 70 l and water flow 120 l h^{-1}. Temperature ranged from 20 to 22°C. *Artemia* biomass (Diet HB) was supplied ad libitum (2–3 times a day) from the first day of culture to the end at 30 days.

2.2.2. *O. vulgaris paralarval culture SS*

The experiment started with a total of 1600 freshly hatched *O. vulgaris* paralarvae divided in four cylindric PVC tanks. Tank volume was 25 l and water flow 80 l h^{-1}. Temperature ranged from 20.6 to 22.5°C. As feeding regime *Artemia* biomass (Diet SS) was supplied ad libitum (2–3 times a day) through the experimental period and pelleted diet from day 10 to 30. Pellets were continuously supplied in excess 24 h day^{-1} using an automatic delivery system (Dohse Aquaristik).

In both experiments illumination was constant 24 h day^{-1}. Tanks were cleaned daily and dead animals removed and counted. At days 10, 20 and 30, samples of 15 paralarvae were cold-anaesthetised (2–4°C) and weighed after blotting on paper to remove the water of the mantle cavity. Dry weights were obtained after drying the samples in an oven for 48 h at 100°C.

2.3. Analytical

Lipids were extracted using the method of Folch et al. (1957). Lipid classes were separated by high performance thin layer chromatography using the method of Olsen and Henderson (1989), and quantified by densitometry (Bio Rad GS 670 Imaging densitometer). For fatty acid analyses, aliquots were transmethylated overnight (Christie, 1982) after the addition of 19:0 as an internal standard (Sigma). Methyl esters were extracted with hexane/diethyl ether (1:1, v/v), and purified by thin layer chromatography (Silica Gel G 60, Merck) using hexane/diethyl ether/acetic acid (85:15:1.5, v/v/v) as solvent. The analyses of the methyl esters were carried out in a Fisons 8000 gas chromatograph equipped with a fused silica 30 m × 0.25 mm open tubular column (Tracer, TR-WAX, film thickness: 0.25 μm) and a cold on-column injection system, using helium as carrier gas, and a thermal gradient from 50 to 220°C. Peaks were

recorded in a personal computer linked to the analytical instrument using Chrom-Card software (Fisons), quantified with the aid of the response factor of the internal standard and identified by comparison with a well characterised marine oil.

3. Results

All the cephalopod paralarvae and juveniles were rich in polar lipid and cholesterol (Chol.), the latter ranging from 18 to 25% of total lipid (Fig. 1). In general, PC, phosphatidylethanolamine (PE) and Chol. accounted for more than 60% of the total lipid. Squid and cuttlefish were richer in PC (34, 33%) and PE (25, 22%) as compared to newly hatched octopuses (21, 16%). The lipids of *O. vulgaris* paralarvae, both newly hatched and cultured were richer in phosphatidylinositol (PI) among the polar lipids, and sterol esters (SE) in the neutral lipid fraction. After culture with Diet HB *Artemia* biomass, the lipid class profile of the octopuses clearly shifted towards the neutral lipids due to an increase in triacylglycerol (TAG).

The main fatty acids of cephalopods were 16:0, 20:5$n-3$ and 22:6$n-3$ (Table 1). Twenty five to 35% of the total fatty acids were saturated, 9 to 14% were monoenes, and approximately 50% were polyunsaturated of which the majority were $n-3$, and longer than 20 C atoms. Although the PUFA content of all the octopuses analysed was similar, squid and cuttlefish were richer in $n-3$ PUFA than octopus, whereas octopus had higher $n-6$ PUFA due to relatively high levels of 20:4$n-6$. Squid paralarvae were particularly low in $n-6$ PUFA and thus showed a very high $n-3/n-6$ ratio. DHA and EPA were also more abundant in squid and cuttlefish hatchlings as compared to octopus paralarvae.

The lipid class profile of the mysids and pagurid zoeae was clearly distinct from the rest of the food items (Fig. 2). In both groups, the phospholipids were particularly abundant, mainly PC and PE (Mysids were also rich in phosphatidylserine (PS) and phosphatidic acid/cardiolipin (PA/CL)), whereas in the *Artemia* diets and pellets, the neutral lipids were more prominent. HB and SS enriched *Artemia* were particularly rich in TAG, the former showing also a high proportion of SE. Ethyl esters (EE) were the most abundant lipid class of pellets together with the free fatty acid fraction (FFA). Chol. was over 10% in all foods. Three distinct fatty acid profiles can be distinguished among the food items analysed: on one side, mysids and zoeae, on the other, enriched *Artemia*, and finally, inert food (Table 2). The natural foods (mysids and zoeae) were very rich in PUFA, mainly $n-3$ fatty acids. Their DHA and EPA content was very high with the fatty acids in a 1:1 ratio. Pagurid zoeae were richer in $n-6$ PUFA compared to the mysids, mainly due to their higher content of arachidonic acid (20:4$n-6$). The fatty acid profiles of both enriched *Artemia* were clearly distinguished by their high content in 18, 20 and 22 C monounsaturated fatty acids, their low levels of DHA and low DHA/EPA ratio. *Artemia* and pellets were rich in 18:2$n-6$ in comparison with mysids and pagurids. The pellets showed higher levels of PUFA than the *Artemia* diets, but their fatty acids were richer in monoenes as compared to mysids and pagurids. Although all food items had a similar proportion of saturated fatty acids

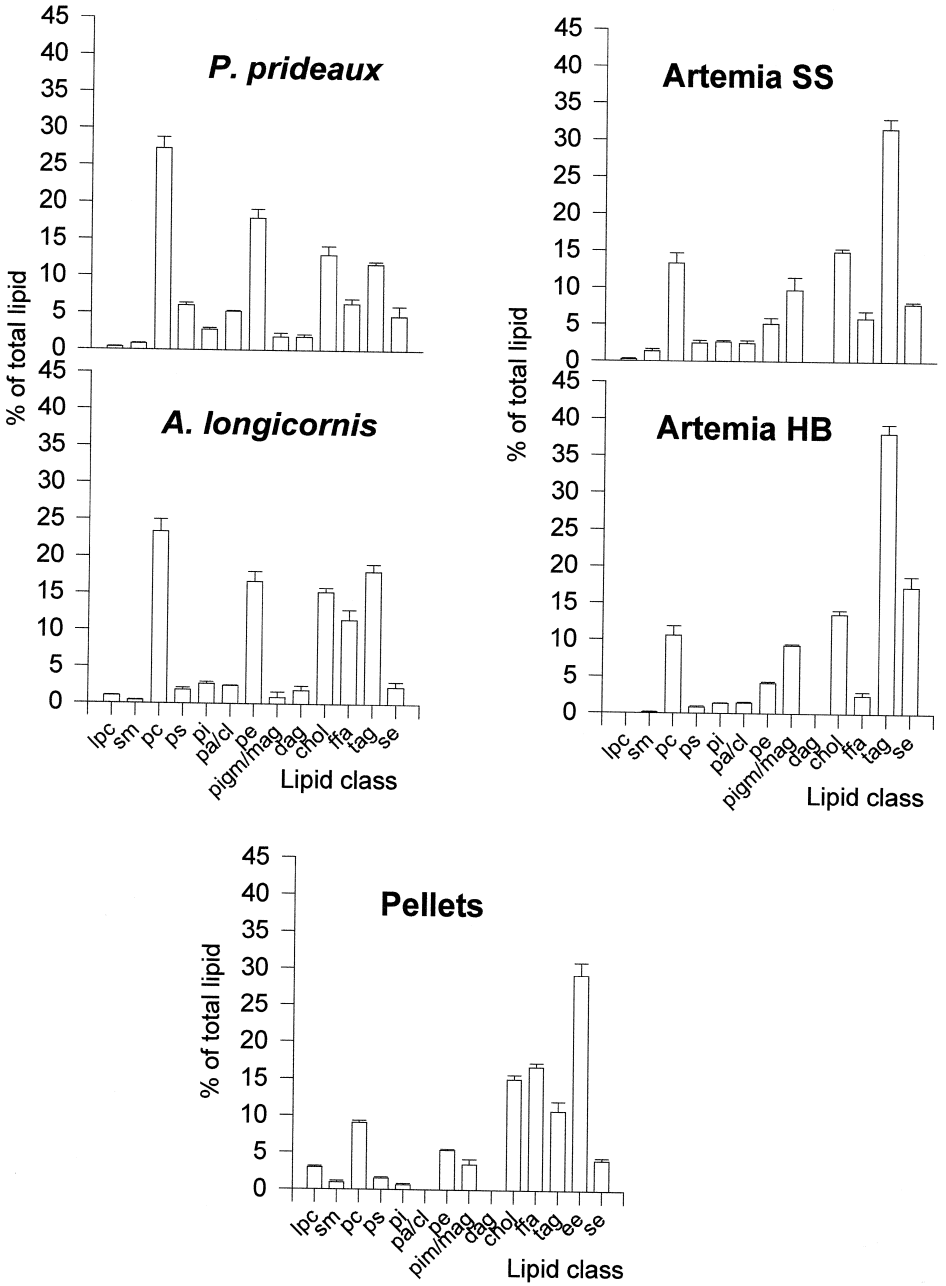

Fig. 2. Lipid class composition (% of the total lipid) of natural food (Mysids (*A. longicornis*), Pagurid zoeae (*P. prideaux*) and feeds (*Artemia* SS and HB, Pellets) used in the rearing of early stages of cephalopods. Data are the mean of four replicates. Error bars are the standard deviation. Abbreviations are like in Fig. 1 plus ee: ethyl esters. For more explanation see the text.

Table 2
Total lipid and total fatty acids (% of dry weight), and fatty acids from total lipid (wt.%) of natural food and feeds used in the rearing of early stages of cephalopods
Data are the means of three replicates. Standard deviations were below 10%.
0.0 are values below 0.09.

Fatty acid	Food items				
	Mysids *A. longicornis*	Pagurid zoeae P. prideaux	Artemia SS	Artemia HB	Pellets
< 14:0	0.8	1.1	0.6	0.4	0.2
14:0	1.4	0.6	1.9	1.5	1.5
14:1	0.2	0.2	0.2	0.3	0.1
15:0	0.8	0.8	0.3	0.4	0.2
15:1	0.2	0.3		0.2	0.0
16:0	22.2	13.8	13.6	12.3	18.4
16:1$n-9$	0.2			1.0	
16:1$n-7$	1.2	2.6	5.9	4.0	2.3
16:1$n-5$	0.2			0.2	
16:2		0.2	0.5	1.2	0.4
17:0		2.4	0.8	1.0	0.3
16:3	0.3	0.9	0.6	0.6	0.2
16:4					0.1
18:0	4.3	7.8	9.5	8.2	7.4
18:1$n-9$	5.8	6.8	21.7	20.5	16.7
18:1$n-7$	4.7	6.2	9.5	8.0	2.0
18:2$n-6$	1.2	1.2	6.2	7.1	6.3
18:3$n-6$		0.2		0.1	0.1
18:3$n-3$	0.5	0.3	3.8	7.0	0.5
18:4$n-3$	0.5		0.4	0.8	0.8
20:0	0.2	0.4	0.3	0.3	0.3
20:1$n-9$	0.7	1.0	5.0	4.8	3.3
20:1$n-7$	0.1	0.9			0.3
20:2$n-6$	1.7	1.3	0.3	0.4	0.3
20:3$n-6$		0.2		0.1	0.3
20:4$n-6$	1.3	5.2	4.0	1.9	2.5
20:3$n-3$	0.3	0.2	0.2	0.4	0.1
20:4$n-3$	0.4	0.2	0.2	0.3	0.5
20:5$n-3$	21.9	15.0	7.1	8.2	12.4
22:0			0.3	0.2	0.1
22:1$n-11$		0.3	3.0	2.8	2.8
22:1$n-9$					0.7
22:2$n-6$	0.3	0.2		0.1	0.6
22:4$n-6$		0.8			0.3
22:5$n-6$	0.4	0.8			0.8
22:3$n-3$					0.1
22:5$n-3$	0.4	1.8	0.4	0.5	2.1
22:6$n-3$	24.0	18.1	1.6	2.3	13.4
Total	96.1	91.7	98.0	96.9	98.4
Saturated	28.9	25.9	26.6	23.9	28.0
Monoenes	13.2	18.2	45.3	41.7	28.3
Polyunsaturated	53.2	46.5	25.5	30.9	41.8
$n-3$	48.1	35.6	13.8	19.4	29.9
$n-6$	4.8	9.9	10.5	9.7	11.2

Table 2 (continued)

Fatty acid	Food items				
	Mysids *A. longicornis*	Pagurid zoeae P. prideaux	Artemia SS	Artemia HB	Pellets
PUFA $n-3$	47.1	35.3	9.6	11.7	28.6
PUFA $n-6$	3.6	8.5	4.3	2.5	4.8
$n-3/n-6$	10.0	3.6	1.3	2.0	2.7
dha/epa	1.1	1.2	0.2	0.3	1.1
% total fatty acids	3.0	5.9	7.6	10.7	16.6
% total lipid	12.3	19.2	24.6	37.7	32.9

(26–29% of total fatty acids), 16:0 was very high in the mysids as compared to all the other feeds.

After 30 days culture of *O. vulgaris* paralarvae, poor growth and survival were achieved (Table 3). During the first 10 days growth was similar in both dietary treatments, then stopped for 10 more days and increased again to give differences between the paralarval cultures fed *Artemia* Diet HB and *Artemia* Diet SS + pellets. Slopes of the growth curves of HB and SS cultures were different (Student's t test, $P < 0.05$). Better growth was achieved in the HB octopuses but, on the other hand, survival was the lowest (Table 3).

When octopus were cultured for 30 days, their fatty acid composition changed and reflected that of the food (Table 1). The effect of the *Artemia* diet was clear in the fact that there was an increase in the content of 18:1 fatty acids, the most abundant fatty acid in *Artemia*. Animals fed pellets, richer in DHA than enriched *Artemia*, showed a higher proportion of this fatty acid as compared with the other group fed solely by enriched *Artemia* Diet HB. *O. vulgaris* paralarvae both newly hatched and cultured were very rich in 20:4$n-6$ compared with the other cephalopods analysed.

O. vulgaris paralarvae adopted aiming postures and forward swimming with extended arms when approaching the pellets, in a behaviour similar to the one observed

Table 3
Mean weight (mg) and survival (%) of *Octopus vulgaris* at 0, 10, 20 and 30 days of paralarval cultures HB and SS
Standard deviations in parentheses.
0.0 are values below 0.09.

Days of culture	Cultures					
	Octopus HB			Octopus SS		
	Wet weight	Dry weight	Survival	Wet weight	Dry weight	Survival
0	1.4 (0.1)	0.3 (0.0)	100	1.4 (0.1)	0.3 (0.0)	100
10	1.5 (0.3)	0.4 (0.1)	11.0	1.6 (0.1)	0.4 (0.0)	33.2
20	1.7 (0.3)	0.5 (0.1)	4.1	1.7 (0.1)	0.4 (0.0)	17.7
30	4.4 (0.9)	1.0 (0.2)	1.5	3.1 (0.3)	0.7 (0.1)	6.7

when they attacked live preys (Villanueva and Nozais, 1996). The pellets were captured when sinking in the water column. No captures were observed on the pellets deposited on the bottom of the tank. Rearing observations on the behaviour of pellet capture and ingestion were done at the age of 15 and 16 days. During six feedings, a minute after the addition of pellets, 100 individuals were counted, and the number of paralarvae with pellets within their arms recorded. The mean percentage of paralarvae handling pellets was 49% (range: 39–64%). Stomach contents were visualized by transparency, since the stomach took the pellet colour when ingestion was successful. A total of 62 paralarvae handling pellets was followed individually by the observer, with 18% of them ingesting the pellet. This process lasted from 65 to 920 s.

4. Discussion

It has been reported that it is particularly difficult to increase the contents of DHA and EPA in enriched *Artemia* nauplii (Dendrinos and Thorpe, 1987; Navarro et al., 1999). It seems even more difficult to increase the levels of both fatty acids in enriched *Artemia* biomass in view of the data reported here and elsewhere (Naessens et al., 1997). The low DHA content in HB and SS enriched *Artemia* would suggest a deficient enrichment technique but could also be the result of the lower volume of the digestive tract-to-body ratio in *Artemia* biomass as compared to newly hatched nauplii.

All the newly hatched cephalopods showed relatively low lipid contents. Low lipid contents, with relatively large phospholipid and sterol fractions and scarce reserve lipids have been hypothesised to be typical of early developmental stages where most of the energy is channelled towards growth (Piatkowski and Hagen, 1994). However, low lipids may be a typical common feature of the flesh of many cephalopods (Strancari-Stockel and Lozano-Soldevila, 1987) and the higher lipid content in one of the cultured paralarvae (Octopus HB) can be considered further evidence of a metabolic imbalance caused by improper food composition (higher lipid content of diet HB).

TAG have been reported as minor components of the flesh of cephalopods (Jangaard and Ackman, 1965; Nash et al., 1978; Hayashi and Yamamoto, 1987). They are, however, relatively abundant in the digestive glands of gonatid (3–44%) (Hayashi and Yamamoto, 1987) and ommastrephid squids (58%) (Nash et al., 1978). On the other hand, Piatkowski and Hagen (1994) found that TAG represented 13 to 26% of the total lipid of cranchid squids. This figure agrees with what we report here for newly hatched *O. vulgaris* and for reared paralarvae of the culture SS, but it is higher when compared with squid and cuttlefish and lower than the values obtained for the HB culture.

Diacylglyceryl ethers and plasmalogens have been described as minor, although significant, constituents of several species of cephalopods including the octopus (de Koning, 1972; Hayashi and Kawasaki, 1985; Hayashi and Yamamoto, 1987; Hayashi, 1989; Hayashi et al., 1990). The analytical protocol used in the present work did not allow us to separate these constituents from other lipid classes (Henderson and Tocher, 1992). Other unusual components, like the ceramide ciliatine, have been described as constituents of the lipids of octopuses (de Koning, 1972). During analysis, these would

migrate close to PI and may not have been fully separated (Henderson and Tocher, 1992) accounting for the high PI of octopus paralarvae.

Palmitic acid (16:0), 20:5$n-3$ and 22:6$n-3$ are the most abundant fatty acids found in the lipids of many cephalopod species, not only at the flesh or whole body level (Jangaard and Ackman, 1965; Culkin and Morris, 1970) but also in the digestive glands (Jangaard and Ackman, 1965; Hayashi et al., 1990), the central nervous system (Dumont et al., 1992; Dumont et al., 1994) and in photoreceptors (Eguchi et al., 1994). In view of this fatty acid composition and the data reported here, one can deduce that the long chain PUFA requirements of cephalopod paralarvae must be very high.

There was a clear influence of the lipid composition of the food on the lipids of cultured octopus paralarvae both at the level of lipid class and fatty acid composition. HB *Artemia* fed octopuses showed a clear increase in TAG, whereas the octopus paralarvae fed pellets showed a lipid class profile closer to the one of the newly hatched individuals. The paralarvae fed pellets had a higher content of free fatty acids, which were abundant, together with ethyl esters, in the pellets. The influence of the lipid composition of the natural food on the body composition of cephalopods both in the flesh and in the digestive gland has also been discussed by other authors (Joseph, 1989).

The dietary requirements for $n-3$ PUFA, particularly DHA is critical in early developmental stages due to the high demand for membrane synthesis where the $n-3$ PUFA are incorporated (Henderson and Sargent, 1985). DHA plays an important role in maintaining the structural and functional integrity of cell membranes in fish (Sargent, 1995). This fatty acid may be even more important for the correct development and survival of fast growing phospholipid-rich cephalopod paralarvae.

The cultured octopus paralarvae showed a lower content of PUFA as compared with the newly hatched individuals. The differences were particularly marked with DHA and EPA, were probably of dietary aetiology in view of the results obtained when fed pelleted diet, and can be the cause of the poor performance of the paralarval rearing.

Fish larvae are strongly dependent on the fatty acids of their prey for the elaboration of major polar lipids in key organs including neural and visual apparatus (Navarro et al., 1993b, 1995). Navarro et al. (1993b) reported that 30 days of dietary manipulation were sufficient to change the fatty acid composition of the lipids of the heads, eyes and bodies of herring larvae. Similar results were obtained with sea bass larvae (Navarro et al., 1995) and with weaned turbot (Mourente et al., 1991) and sea bream (Mourente and Tocher, 1993). In view of the data reported here, this dietary effect seems to be a fast generalised process not limited to fish and crustacean (Montaño and Navarro, 1996) larvae, whose physical (poor growth, survival and resistance) and physiological significance (Bell et al., 1995) can perhaps be extrapolated to cephalopod paralarvae.

Despite the better lipid class profile and fatty acid composition of group SS, low survival and growth were reached. Since the lower survival of this group was obtained from the beginning of the culture, causes other than the quality of food may be directly involved. In fact, the clogging of the filters at day 8 and a decrease in water quality due to an excess of pellets offered to the paralarvae at day 10 seem to be the causes of peak moralities in cultures HB and SS, respectively. Aside from these considerations, both cultures gave poor growth and survival as compared to other cultures carried out using crustacean zoeae as food (see Villanueva, 1995), pointing to causes other than simply

the lipid composition of food for the lack of success in this paralarval culture, perhaps a strong dietary imbalance of the enriched *Artemia* and difficulties in getting the paralarvae to accept inert food.

During the paralarval rearing, it was observed that the paralarvae fed on the pelleted diet. With our experimental design it was impossible to quantify the amount of inert food ingested but it is clear that this had an effect on the lipid composition of paralarvae, which encourages the use of co-feeding techniques to correct the deficient nutrient composition of *Artemia* as sole paralarval food (Rosenlund et al., 1997). This is the first time that pellets have been used for rearing paralarval stages of cephalopods. In contrast with larval fishes, cephalopod paralarvae manipulate the food with their arms before ingestion after capture. The high incidence of captured pellets by *O. vulgaris* paralarvae but with subsequent low ingestion indicates that palatability could be a determinant factor on the ingestion rates. Although during the present study, low success was obtained with pellet ingestion, co-feeding techniques can be a way in the future for rearing cephalopod paralarvae, provided palatability and feeding protocols are improved. Pelleted diets have been previously used for rearing subadult stages, indicating the adaptability of cephalopods to feed on inert food (Lee et al., 1991; Castro et al., 1993; Castro and Lee, 1994).

5. Conclusion

In view of the present results, *Artemia* does not seem to be the right choice to feed early stages of cephalopods. From their lipid composition and that of their natural food, it can be deduced that cephalopod paralarvae and juveniles must require a food rich in PUFA, phopholipids and cholesterol and with a moderate content of neutral lipids. This is particularly clear for the squid *L. vulgaris* and the cuttlefish *S. officinalis*. However, these theoretical lipid requirements are common to most fish and crustacean larvae that, on the other hand, are reared with different degrees of success using enriched *Artemia* nauplii. Fine tuning of enrichment by using enrichment diets high in polar lipids (McEvoy et al., 1996) and PUFA and/or co-feeding enriched *Artemia* with inert diets similar to the pellets used in the present experiments should be the choice to attempt paralarval rearing of cephalopods with minimum guarantees of success.

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