Composition in essential and non-essential elements of early stages of cephalopods and dietary effects on the elemental profiles of *Octopus vulgaris* paralarvae

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Abstract

During the present study, we aimed at providing a first look at the elemental composition of the early stages of cephalopods as an approach to their elemental requirements in culture. Essential and non-essential elemental profiles of the European cuttlefish *Sepia officinalis*, the European squid *Loligo vulgaris* and the common octopus *Octopus vulgaris* laboratory hatchlings and wild juveniles were analysed. In addition, for *O. vulgaris* we determined elemental profiles of mature ovary, eggs in different stages of development and followed possible effects of four dietary treatments during paralarval rearing, also analyzing elemental content of the live preys *Artemia* nauplii and *Maja brachydactyla* hatchling zoeae. Content was determined for essential (As, Ca, Cr, Co, Cu, Fe, K, Mg, Mn, Na, Ni, P, Rb, S, Sr, Zn) and non-essential (Ag, Al, Ba, Cd, Hg, Pb) elements. The content in non-essential elements found in hatchlings and juveniles of the three species analyzed here seems to be far lower in comparison with subadult and adult stages of coastal cephalopods. In the octopus eggs, the non-essential element concentrations remained globally low compared to hatchlings and juveniles indicating the absorption of these elements along the ontogenetic development. The elemental composition of the octopus ovary and of the eggs, hatchlings and juveniles of the three cephalopod species analyzed here showed a high content in S. As expected, the calcified internal shell of the cuttlefish, rich in Ca and Sr, originates the main difference between species. It is remarkable the richness in Cu of hatchling octopus, that may indicate a particular nutritional requirement for this element during the planktonic life. The reared octopus paralarvae feed on *Artemia* nauplii, a prey with relatively low Cu content, showed nearly half Cu content that the "natural" profile of octopus hatchlings or wild juveniles. This suggests a dietary effect and/ or an indication of the poor physiological stage of the *Artemia*-fed paralarvae. At the present, the percentage of essential element absorption by food or seawater is unknown for cephalopods and should be determined in the future to understand their feeding requirements in culture.
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Keywords: Biochemical composition; Eggs; Larvae; *Sepia*; *Loligo*; *Octopus*

1. Introduction

Minerals are required for the maintenance of normal metabolic and physiological functions of living organisms. The main functions of essential elements in the

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animal body include the formation of skeletal structure, maintenance of colloidal systems, regulation of acid–base equilibrium and they are important components of hormones, enzymes and structural proteins (e.g. Simkiss, 1979; Williams, 1981; Lall, 2002). The present knowledge of the elemental composition of cephalopods mainly comes from subadult and adult forms and has focused on selected organs or body portions (see between others, Miramand and Bentley, 1992; Bustamante et al., 2000; Ichihashi et al., 2001a; Napoleao et al., 2005a). Most of these studies have highlighted the very high ability of cephalopods to concentrate various toxic elements such as Ag or Cd (e.g. Martin and Flegal, 1975; Bustamante et al., 1998a, 2002a, 2004) and radionuclides such as ^{241}Am, ^{60}Co, ^{137}Cs, ^{210}Po and ^{237}Pu (Suzuki et al., 1978, Guary et al., 1981; Smith et al., 1984; Yamada et al., 1999).

Cephalopods are carnivorous, active predators and the environmental induced toxic elements have been the subject of recent research regarding detoxification processes (Tanaka et al., 1983; Finger and Smith, 1987; Castillo et al., 1990; Castillo and Maita, 1991; Craig and Overnell, 2003; Bustamante et al., 2002b). However, the elemental requirements of this group of molluscs are poorly known and few studies have been done in relation with the elemental content of early stages of cephalopods and their possible role for the development of embryos and growth of paralarvae and juveniles (Decleir et al., 1970; Miyazaki et al., 2001). For example, it clearly appears that Sr is of ground importance for the shell and statolith development and thus normal swimming behaviour and survival of hatchling cephalopods (Hanlon et al., 1989).

Because cuttlefish are among the easier cephalopod species to rear, several experimental investigations have been carried out on the incorporation of trace elements by their eggs. These studies have shown that the eggshell prevents the incorporation of some non-essential metals such as Cd, Pb, or V and of essential Cu and Zn as well (Paulij et al., 1990; Bustamante et al., 2002a; Miramand et al., 2006). But at the same time, other elements such as Ag and Cs can pass through the eggshell and become incorporated in embryonic tissues (Bustamante et al., 2004, 2006). Element transport selectivity through the eggshell is apparently not determined by the metabolic needs of the embryo for essential elements, since the non-biologically essential element Ag is well known for its enhanced embryotoxicity (Calabrese et al., 1973; Martin et al., 1981; Warnau et al., 1996). By another hand, no information exist on the incorporation of elements by eggs in cephalopod species that lack eggshell, as in the incirrate octopods (in ex., *Octopus vulgaris*) which egg

chorion is in direct contact with seawater. Overall, after the hatchling, accumulation of toxic elements shows two patterns with 1) metals such as Ag which is accumulated immediately since juveniles are in direct contact with seawater 2) metals such as Cd or Pb which are significantly incorporated only once the cephalopods start to feed (Miramand et al., 2006). After first feeding most part of the elements can be assumed to be incorporated from the diet and it is known that in juvenile cuttlefish *Sepia officinalis* the diet influences the elemental composition of the calcareous statoliths (Zumholz et al., in press). However, the behaviour of most of essential elements remains poorly understood to date according to the bioaccumulation processes or to the nutritional needs of cephalopod paralarvae and juveniles.

Due to their rapid growth and market price, the culture of cephalopods has been an increasing area of interest (Walsh et al., 2002; García García et al., 2004; Nabhitabhata et al., 2005; Sykes et al., 2006). However, the rearing of the delicate early stages seems to be the main bottleneck to develop the aquaculture of some species such as *S. officinalis* (Domingues et al., 2001, 2003; Koueta et al., 2002; Koueta and Boucaud-Camou, 2003) and *O. vulgaris* (Itami et al., 1963; Villanueva, 1994, 1995; Carrasco et al., 2003; Iglesias et al., 2004; Okumura et al., 2005). The artificial feeding of the early stages of cephalopods is an unresolved problem and to the present only cultures at experimental scale using natural prey has been successful. Aside from the problems related to food size and quantity, there seem to be other problems associated with food quality. Previous studies on the biochemical composition of the early stages of cephalopods have been developed as first approaches to determine their feeding requirements for lipids and amino acids, trying to design possible co-feeding techniques using *Artemia* and microdiets suitable for the paralarval feeding behaviour (Villanueva et al., 1995, 1996, 2004; Rosenlund et al., 1997; Hernández-García et al., 2000; Navarro and Villanueva, 2000, 2003). The present work follows this research topic aiming at taking a first insight on the elemental requirements of the paralarval and juvenile stages of cephalopods in culture.

First, we determined the elemental composition of laboratory hatchlings and wild juveniles of three shallow water cephalopod species that represent the main cephalopod orders, all of them of high commercial interest: the European cuttlefish *S. officinalis*, the European squid *Loligo vulgaris* and the common octopus, *O. vulgaris*. Second, for *O. vulgaris* we determined the same elemental profiles in mature ovary, eggs in different stages of development, hatchlings fasted during 4 days, paralarvae reared to 20 days with four dietary treatments, and the

Artemia nauplii used as food during these experiments. In addition, we analyzed hatchling zoeae of the spider crab *Maja brachydactyla*, a prey that has been successfully used previously as food resource for rearing *O. vulgaris* during the planktonic stage (Carrasco et al., 2003; Iglesias et al., 2004).

Following Mason and Jenkins (1995), we divided here the analyzed elements as essential elements (As, Ca, Cr, Co, Cu, Fe, K, Mg, Mn, Na, Ni, P, Rb, S, Sr, Zn) and non-essential elements (Ag, Al, Ba, Cd, Hg, Pb). Even Sr is generally reported as a non-essential element for biota, it is essential for cephalopods according to Hanlon et al. (1989) (see above). The knowledge about the essential character and function of each element in cephalopods is poorly known and this classification may change according with future research.

2. Materials and methods

2.1. Collection of material

2.1.1. Cephalopod hatchlings and wild juveniles

Specimens analyzed here were used also to obtain their amino acid composition in a previous published study (Villanueva et al., 2004) where detailed information on the collection of material is indicated. In short, egg masses of *S. officinalis* and *L. vulgaris* were collected off Barcelona (NW Mediterranean) and egg masses of *Octopus vulgaris* were obtained from a broodstock maintained in the Institut de Ciències del Mar (ICM), Barcelona. Healthy individuals of all three species were preserved during the first 24 h after hatching in the laboratory. The samples were collected using a hand net, washed in tap water, then placed on blotting paper to remove the excess water, weighed on a microbalance, frozen at −80 °C and freeze-dried overnight. The dry weight was obtained from the freeze dried samples, which were then stored again at −80 °C for subsequent elemental analysis (see below). To determine wild juvenile elemental profiles, 6 *S. officinalis* wild juveniles (25.9–103.8 g wet weight) collected from the artisanal fishery off Cambrils (Tarragona, NW Mediterranean); 5 *L. vulgaris* juveniles (2.0–2.8 g wet weight) collected from the local trawl fishery off Barcelona (NW Mediterranean) and 5 benthic *O. vulgaris* juveniles (3.5–14.2 g wet weight) captured from the wild by scuba diving off L'Estartit (NW Mediterranean) were analyzed. All juveniles were weighed fresh and frozen at −80 °C upon arrival in the laboratory. All wild juveniles were freeze-dried, with exception of *S. officinalis* individuals, which were dried at 60 °C in an oven during 7 d to constant weight.

2.1.2. Rearing experiments of O. vulgaris paralarvae

Specimens analyzed here belong to the same culture experiments reported also in Villanueva et al. (2004) were detailed information on rearing methods can be found. Four experimental treatments were used (see below) and each treatment was conducted in quadruplicate. Paralarvae were reared for 30 d using cylindrical 25-l volume PVC tanks at mean temperature of 20.4 °C (range 19.2–21.1 °C). In all experiments, on day 20, all individuals in each rearing tank were counted and transferred to an identical clean tank. The percentage survival (S) was calculated as $S = 100S \cdot (I–B)^{-1}$, where S was the number of surviving individuals on day x, I was the initial number of individuals in the culture, and B was the total number of individuals killed for sampling purposes to day x. On day 20, to determine growth and for subsequent analysis (see below), 6 samples of 20 paralarvae each were collected from each rearing experiment. Paralarval samples were collected 2 h after the first daily food addition (see below), washed in tap water, placed over a plastic mesh on blotting paper to remove excess water, stored in Eppendorf tubes, weighed, frozen at −80 °C and freeze-dried overnight. The dry weight was obtained from the freeze-dried paralarvae, which were stored again at −80 °C for subsequent elemental analysis (see below).

2.1.3. Feeding treatments of O. vulgaris paralarvae

All treatments were fed enriched *Artemia* nauplii (AF, INVE Aquaculture) 450 μm in length, which were provided from day 0 to day 20 at a ration of 6–7 nauplii ml^{-1}d^{-1}. *Artemia* nauplii were enriched in seawater for 24 h at 28 °C with one of the following enrichment diets: a) Diet SS: DC Super Selco (INVE) 0.6 g l^{-1}, and, (b) Diet MET: DC Super Selco 0.6 g l^{-1} and 0.8 g l^{-1} of L-methionine (Sigma Products). To test the influence of the presence of amino acids in seawater, essential L-amino acids in crystalline form (Sigma Products) were added to the rearing tanks (see Villanueva et al., 2004 for details) and four treatments were tested, 1) Control group: paralarvae were fed *Artemia* nauplii enriched with Diet SS; 2) MET group: paralarvae were fed *Artemia* nauplii enriched with Diet MET; 3) AA group: paralarvae were fed *Artemia* nauplii enriched with Diet SS and they also received a daily amino acids solution in the rearing tank, and, 4) METAA group: paralarvae were fed *Artemia* nauplii enriched with Diet MET and they also received a daily amino acids solution in the rearing tank. An unfed group was also maintained from the hatchling stage to day 4.

2.1.4. Paralarval preys

The elemental compositions of *Artemia* nauplii from the Diet SS and Diet MET groups, as well as the elemental

composition of recently hatched zoeae of spider crab *M. brachydactyla* were analysed. These zoeae (not used as food during the present study) have been used previously with success as a food resource for rearing *O. vulgaris* (Carrasco et al., 2003; Iglesias et al., 2004) during the first 2 months of paralarval life. Samples were collected and preserved as described for the cephalopod paralarvae.

2.1.5. Mature ovary and eggs of O. vulgaris

Ovary samples were obtained from a wild mature female, 2900-g total fresh weight, collected off Barcelona. Eggs of stage I–II and X–XII (Naef, 1928) were collected from egg masses obtained in the laboratory.

2.2. Analytical

2.2.1. Elemental profiles

Elemental analyses were carried out at the Serveis Cientificotècnics, Universitat de Barcelona. All elemental analyses were made from three 25 mg dry weight aliquotes. Homogenized dry samples were digested with 1 ml HNO_3 and 1 ml H_2O_2 ("Baker Instra") in 60 ml teflon reactors (Savillex, catalog number 561 R2, Techmate, UK) overnight at 90 °C. After digestion, 15 ml deionized H_2O (Milli-Q quality), was added and final weight obtained. Density of the sample was calculated by weight of 5 ml of digested solution. Samples were digested by triplicate with 8 blanks in each batch of analysis.

Determination of Ca, Fe, K, Mg, Na, P and S were obtained by inductively coupled plasma (ICP-AES) using a Perkin Elmer Optim 3200 RL multichannel analyzer calibrated with 5 standards in 5% HNO_3. Digested samples were analyzed without dilution. Determination of Ag, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Rb, Sr, and Zn were obtained by inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin Elmer ELAN 6000 analyzer, calibrated with 5 standards in 1% HNO_3. Rhodium was used as internal standard. Digested samples were analyzed without dilution, with Rh addition by flow injection analysis system.

Reference materials from the Community Bureau of Reference (BCR) of the Commission of the European Communities, Plankton CRM 414 and Cod Muscle CRM 422, were treated and analysed in the same way. Results for the standard reference materials were in good agreement with certified values and the mean recovery of all elements was of $99 \pm 15\%$ (range: 73–128%).

2.3. Data treatment

Mean values (after arcsinus-transformation for survival data) were compared by the Student *t*-test and

Table 1
Means±SD of the wet and dry weights (in mg ind^{-1}), and the essential and non-essential elemental content (in µg g^{-1} of dry weight) of *Sepia officinalis*, *Loligo vulgaris* and *Octopus vulgaris* hatchlings

	Sepia officinalis	*Loligo vulgaris*	*Octopus vulgaris*
Wet weight	82.1±5.3	3.5±0.1	2.1±0.1
Dry weight	20.8±1.0	0.8±0.0	0.3±0.0
Major essential elements			
Ca	12158±1055[a]	1562±50[b]	2496±52[b]
K	15363±1518[b]	18426±324[a]	17801±429[a]
Mg	2342±408[b]	1720±24[c]	3270±126[a]
Na	13792±1387[c]	8392±251[b]	17815±585[a]
P	11311±519[b]	13103±90[a]	13382±279[a]
S	22980±1650[b]	26077±342[a]	28647±761[a]
Minor essential elements			
As	144±4[a]	53.0±0.4[c]	78.6±1.8[b]
Cr	<2	<2	<2
Co	<0.07	<0.07	<0.07
Cu	58.6±10.7[b]	69.8±1.9[b]	217±3.0[a]
Fe	19±2[a]	23±2[a]	21±1[a]
Mn	1.9±0.1[b]	1.6±0.0[c]	3.7±0.1[a]
Ni	<0.5	<0.5	1.3±0.1
Rb	5.8±0.5[b]	8.1±0.1[a]	7.9±0.2[a]
Sr	107±10[a]	24.8±0.8[c]	43.8±1.3[b]
Zn	101±10[b]	105±2[b]	182±5[a]
Non-essential elements			
Ag	0.5±0.2[b]	0.8±0.0[b]	2.8±0.1[a]
Al	10.2±4.0[a]	10.9±2.6[a]	<10
Ba	0.2±0.0	<0.1	<0.1
Cd	<0.07	<0.07	<0.07
Hg	0.3±0.0[a]	0.1±0.0[b]	0.2±0.0[a]
Pb	0.3±0.1[b]	0.3±0.1[b]	0.4±0.0[a]

Elemental analyses were made from at least three replicates. Means±SD with same superscript letters for the same element, denotes no statistical differences within the species ($P>0.05$).
0.0 are values below 0.05.

analysis of variance, followed by the Tukey–Kramer HSD test. Differences were considered significant when $P<0.05$. Data were assessed using the JMP statistical package.

3. Results

Elemental composition of *S. officinalis*, *L. vulgaris* and *O. vulgaris* hatchlings are shown in Table 1. Sulphur, Na, K, P, and Mg were the main elements present in the three species. The calcified, large internal shell of the cuttlefish originates the main structural difference among species. As a result, levels of Ca in *S. officinalis* hatchlings reached more that 5 times that of the other species. Consistently, Sr also showed the higher content as this element has a close behaviour as Ca. In *O. vulgaris*, levels of Ag, Cu, Mn, Ni and Zn were relatively high, reaching

Table 2
Means±SD and range of the wet and dry weigths (in mg ind^{-1}), and the essential and non-essential elemental content (in µg g^{-1} of dry weight) in six individuals of *Sepia officinalis*, and five individuals of *Loligo vulgaris* and *Octopus vulgaris* wild juveniles

Sepia officinalis	Mean±SD	range	R^2	P	Comparison with hatchling content
Wet weight	63643±32993	25870–103757			
Dry weight	15880±8490	6265–26285			
Major essential elements					
Ca	41529±2964	37972–46956	0.10	0.19	High in juveniles
K	13390±399	12816–14242	−0.00	0.79	High in hatchlings
Mg	3509±485	2880–4571	0.00	0.94	High in juveniles
Na	16827±640	15736–17780	**−0.22**	**0.05**	High in juveniles
P	9728±372	9136–10412	−0.03	0.50	High in hatchlings
S	23260±780	21611–24663	0.13	0.14	N.S.
Minor essential elements					
As	70.6±9.5	59.2–83.6	0.00	0.94	High in hatchlings
Cr	<10				
Co	0.9±0.1	0.6–1.0	0.00	0.81	High in juveniles
Cu	190±37	131–242	**0.30**	**0.02**	High in juveniles
Fe	336±272	90–869	**−0.71**	**0.00**	N.S.
Mn	5.7±3.8	2.4–13.5	**−0.66**	**0.00**	N.S.
Ni	<5				
Rb	5.5±0.4	5.0–6.1	−0.53	0.00	N.S.
Sr	354±31	315–422	**0.26**	**0.03**	High in juveniles
Zn	146±19	118–183	**0.51**	**0.00**	High in juveniles
Non-essential elements					
Al	183±160	24.9–485	**−0.78**	**0.00**	N.S.
Ag	3.1±0.8	1.9–4.7	−0.12	0.16	High in juveniles
Ba	2.9±1.8	1.0–6.9	**−0.58**	**0.00**	High in juveniles
Cd	0.9±0.2	1.0–6.9	0.03	0.48	High in juveniles
Hg	0.5±0.2	0.3–0.9	−0.12	0.16	High in juveniles
Pb	1.1±0.4	0.6–1.9	**−0.78**	**0.00**	High in juveniles
Loligo vulgaris	Mean±SD	Range	R^2	P	Comparison with hatchling content
Wet weight	2306±372	1993–2779			
Dry weight	472±86	394–571			
Major essential elements					
Ca	1776±729	1106–3450	−0.03	0.54	N.S.
K	12464±612	11538–13546	**0.68**	**0.00**	High in hatchlings
Mg	3040±263	2706–3511	−0.10	0.25	High in juveniles
Na	19087±1961	16604–22555	−0.26	0.05	High in juveniles
P	10963±415	10170–11487	0.26	0.05	High in hatchlings
S	22859±1074	20821–24510	0.27	0.04	High in hatchlings
Minor essential elements					
As	15.3±1.7	12.9–17.2	−0.06	0.37	High in hatchlings
Cr	<2				
Co	<0.07				
Cu	49.1±6.3	38.8–58.4	0.03	0.52	High in hatchlings
Fe	74±30	32–132	**0.38**	**0.01**	High in juveniles
Ni	<0.5				
Mn	2.4±0.5	1.6–3.5	0.21	0.09	High in juveniles
Rb	4.9±0.4	4.2–5.5	**0.89**	**0.00**	High in hatchlings
Sr	22.8±10.9	13.7–47.5	−0.04	0.48	N.S.
Zn	60.4±3.7	54.0–67.5	0.01	0.80	High in hatchlings

(continued on next page)

Table 2 (*continued*)

Loligo vulgaris	Mean±SD	Range	R^2	P	Comparison with hatchling content
Non-essential elements					
Ag	1.0±0.2	0.6–1.4	**−0.37**	**0.02**	N.S.
Al	56.9±27.8	19.4–103	**0.33**	**0.03**	High in juveniles
Ba	1.4±1.3	0–3–3.9	−0.12	0.21	High in juveniles
Cd	0.3±0.0	0.2–0.3	0.01	0.72	High in juveniles
Hg	0.2±0.0	0.1–0.2	−0.09	0.35	N.S.
Pb	0.6±0.2	0.4–1.1	0.07	0.34	High in juveniles
Octopus vulgaris	Mean±SD	Range	R^2	P	Comparison with hatchling content
Wet weight	7846±4553	3305–14188			
Dry weight	1836±1145	814–3671			
Major essential elements					
Ca	2180±886	1103–3657	0.03	0.57	N.D.
K	14007±1159	11790–16791	0.13	0.18	high in hatchlings
Mg	3420±225	2963–3728	0.06	0.36	N.D.
Na	22217±2367	19074–26485	0.03	0.57	high in juveniles
P	8342±554	7179–9631	**0.39**	**0.01**	high in hatchlings
S	26389±1883	23241–30566	**0.29**	**0.04**	N.D.
Minor essential elements					
As	104±18	74–134	0.03	0.55	high in juveniles
Cr	<5				
Co	0.9 ±0.3	0.5–1.7	**−0.41**	**0.01**	high in juveniles
Cu	159±41	108–229	−0.17	0.14	high in hatchlings
Fe	151±43	83–238	0.00	0.92	high in juveniles
Mn	3.8±1.1	2.3–5.5	−0.00	0.94	N.D.
Ni	1.4±0.4	0.9–2.1	−0.25	0.07	N.D.
Rb	5.5±0.4	4.8–6.3	0.09	0.28	high in hatchlings
Sr	26.2±7.7	15.9–36.9	0.00	0.97	high in hatchlings
Zn	135±22	111–194	−0.25	0.07	high in hatchlings
Non-essential elements					
Ag	2.1±0.5	1.4–3.1	−0.02	0.66	high in hatchlings
Al	67.7±31.0	27.2–119	−0.16	0.22	high in juveniles
Ba	0.6±0.5	0.2–2.2	−0.08	0.37	high in juveniles
Cd	1.7±0.6	1.2–3.7	**−0.40**	**0.02**	high in juveniles
Hg	0.3±0.1	0.2–0.5	−0.07	0.36	N.D.
Pb	1.7±1.4	0.6–6.3	−0.12	0.23	N.D.

Elemental analysis were made from at least three replicates for each individual, with the exception for an *O. vulgaris* specimen 3671 mg dry weight, with only two replicates. Correlation (R^2) between dry weight and concentration of the element is indicated and probability values (P) with significant correlations are indicated in bold face. Elemental content in these wild juveniles in comparison with hatchling content is also indicated. N.S., not significant differences, $P > 0.05$.
0.0 are values below 0.05.

Ag and Cu 5 and 3 times that recorded in other species, respectively. Some differences in other elements were observed and only Fe, Hg and Pb contents were found fairly constant in hatchlings of the three species.

Elemental composition in juvenile wild individuals of the three species is showed in Table 2. In comparison with hatchlings, wild juveniles of the three species increased their content in Ba, Cd, and Na and decreased in K and P. A positive correlation between juvenile dry weight and elemental content was found for Cu, Sr and Zn in *S. officinalis*; Al, Fe, K and Rb in *L. vulgaris*; and P and S in *O. vulgaris*.

In *O. vulgaris*, a notable increase in Ag, Ca, Cu, K, Mg, Na and Sr were observed when comparing the mature ovary with the spawned eggs. These elements increased again in the hatchlings, with exception of the Ag (Table 3). In comparison with recently spawned eggs, the developing *O. vulgaris* eggs have higher concentrations in all the major essential elements, most of the minor essential elements and also some of the non-

Table 3
Means±SD of the essential and non-essential elemental content (in µg g^{-1} of dry weight) of mature ovary, spawned eggs at stages I–II and X–XII, hatchlings and hatchlings fasted 4 d of *Octopus vulgaris*

Octopus vulgaris	Mature ovary	Eggs stage I–II	Eggs stage X–XII		Hatchlings 0 d	Hatchlings fasted 4 d
				Dry weight	337.9±10.1[a]	244±5.9[b]
Major essential elements						
Ca	247±22[b]	278±19[b]	849±2[a]		2496±52[b]	3146±70[a]
K	3545±108[b]	1252±114[c]	8339±24[a]		17801±429[a]	15063±213[b]
Mg	829±62[b]	939±102[b]	2030±17[a]		3270±126[a]	3327±62[a]
Na	5645±301[b]	4857±663[b]	10283±146[a]		17815±585[a]	18620±350[a]
P	8581±132[a]	7106±744[b]	8499±122[a]		13382±279[a]	11604±131[b]
S	19565±276[b]	19350±2054[b]	23369±77[a]		28647±761[a]	29597±444[a]
Minor essential elements						
As	67.6±0.4[a]	52.3±5.8[b]	33.4±0.9[c]		78.6±1.8[b]	88.2±1.1[a]
Cr	<2	<2	<2		<2	<2
Co	<0.07	<0.07	<0.07		<0.07	0.5±0.0
Cu	38.2±0.5[b]	44.2±5.1[b]	86.3±2.0[a]		217±3.0[a]	205±3.9[b]
Fe	26±2[a]	10±1[c]	17±2[b]		21±1[b]	31±1[a]
Mn	3.0±0.1[a]	2.0±0.2[b]	3.3±0.1[a]		3.7±0.1[a]	2.7±0.0[b]
Ni	<0.5	<0.5	1.1±0.1		1.3±0.1[a]	1.3±0.0[a]
Rb	2.0±0.0[b]	1.2±0.1[c]	3.8±0.1[a]		7.9±0.2[a]	6.3±0.1[b]
Sr	4.2±0.4[b]	4.7±0.5[b]	13.3±0.5[a]		43.8±1.3[b]	54.8±1.2[a]
Zn	95.2±1.7[a]	72.7±7.9[b]	102±2.0[a]		182±5.1[b]	371±8.4[a]
Non-essential elements						
Ag	2.3±0.1[b]	2.2±0.3[b]	12.3±0.5[a]		2.8±0.1[b]	11.3±3.1[a]
Al	<10	<10	<10		<10	<10
Ba	<0.2	<0.2	0.2±0.0		<0.1	0.6±0.0
Cd	<0.07	0.1±0.0	<0.07		<0.07	0.3±0.0
Hg	0.3±0.0[a]	<0.14	0.1±0.0[b]		0.2±0.0[b]	0.3±0.0[a]
Pb	0.4±0.0[b]	0.2±0.0[c]	0.6±0.0[a]		0.4±0.0[b]	2.1±0.0[a]

Dry weights (in µg ind^{-1}) in hatchlings correspond to the means±SD. Elemental analyses were made from at least three replicates. Means±SD with same superscript letters for ovary and eggs, and for hatchlings and fasted for dry weight and for the same element, denotes no statistical differences within the group ($P>0.05$).
0.0 are values below 0.05.

essentials as Ag and Pb (Table 3). After 4 d of fasting, hatchlings lost 28% of their dry weight, decreasing their content in Cu, K, Mn, P and Rb, and increasing their levels of Ag, As, Ca, Fe, Sr and Zn (Table 3).

In *O. vulgaris* paralarvae after 20 d of rearing, no significant differences were found for Ag, Ba, Ca, Co, Fe, K, Rb, Sr and Zn contents between the four feeding treatments. Groups that reached the low survival (Control and MET groups, survival of 13–17%) have lower levels of Mg and S than groups with higher survival (AA and METAA groups, survival of 41–54%) (Table 4). Arsenic and Cu reach also the higher levels on the group with best survival (METAA group, Table 4); however, all reared individuals decrease their Cu levels to nearly half percent of the hatchling Cu content, decreasing also As. In contrast, Zn increased notably from the hatchling levels as Ag, Ba, Cd, and Pb. These differences were maintained when comparing with the wild juveniles. In this way, the elemental profile of the reared paralarvae from the Control group, in comparison with the "natural" profile of the hatchlings and wild juveniles, showed low content in As, Cu, Mg and S, and high content in Ag and Zn (Figs. 1, 2 and 3).

Elemental composition of the preys, *Artemia* and *Maja* zoeae, are shown in Table 5. The main element in both species was Na, with higher content in the spider crab zoeae. Elemental profiles of both preys differ notably. Concentration of Ca and Sr in *Maja* were nearly 40 times higher than that of *Artemia*, and Cu and Mg were also 8 and 5 times respectively higher in *Maja*. As expected, enrichment of *Artemia* by methionine (Artemia MET nauplii) resulted in a higher *S* content in these nauplii due to the S richness of this amino acid. However, these enriched *Artemia* do not reach the S content of the *Maja* zoeae. By another hand, *Artemia* nauplii were rich in Fe and Ni. No differences between

Table 4
Means ± SD of wet weight (in mg), dry weight (in µg), survival (in %) and essential and non-essential elemental content (in µg g^{-1} of dry weight) during rearing experiments of *O. vulgaris* at the age of 20 d in four feeding treatments: Control, MET, AA, and METAA (see Materials and methods for details)

Octopus vulgaris	Control	MET	AA	METAA
Wet weight	3.4 ± 0.1[a]	3.5 ± 0.1[a]	3.2 ± 0.2[b]	2.8 ± 0.2[c]
Dry weight	682.8 ± 15.4[a]	681.6 ± 28.4[a]	653.6 ± 20.7[a]	566.2 ± 24.5[b]
Survival	12.6 ± 3.2[b]	17.2 ± 11.2[b]	41.2 ± 9.9[a]	54.1 ± 4.6[a]
Major essential elements				
Ca	1784 ± 42[a]	1840 ± 356[a]	1972 ± 25[a]	2052 ± 108[a]
K	14533 ± 220[a]	14681 ± 527[a]	14659 ± 141[a]	14050 ± 217[a]
Mg	2762 ± 6[c]	2725 ± 65[c]	3078 ± 52[a]	2900 ± 41[b]
Na	15035 ± 410[b,c]	14501 ± 839[c]	17255 ± 599[a]	16411 ± 338[a,b]
P	11321 ± 112[a,b]	11579 ± 262[a]	11439 ± 189[a,b]	10805 ± 383[b]
S	19996 ± 150[c]	21418 ± 651[b]	23135 ± 123[a]	22691 ± 112[a]
Minor essential elements				
As	44.6 ± 1.4[c]	47.8 ± 0.9[a,b]	46.8 ± 0.2[b,c]	50.4 ± 1.0[a]
Cr	<2	<2	<2	<2
Co	0.4 ± 0.0[a]	0.4 ± 0.0[a]	0.4 ± 0.0[a]	0.4 ± 0.0[a]
Cu	91.7 ± 3.0[b]	96.5 ± 4.5[a,b]	97.4 ± 0.7[a,b]	104 ± 5.3[a]
Fe	71 ± 3[a]	72 ± 6[a]	79 ± 12[a]	72 ± 1[a]
Mn	3.4 ± 0.2[b]	3.6 ± 0.1[a,b]	3.8 ± 0.0[a]	3.4 ± 0.0[b]
Ni	1.0 ± 0.0[a]	0.7 ± 0.1[b]	0.7 ± 0.1[b]	0.5 ± 0.0[c]
Rb	6.6 ± 0.3[a]	6.8 ± 0.2[a]	6.9 ± 0.0[a]	6.6 ± 0.1[a]
Sr	30.4 ± 1.7[a]	31.3 ± 5.1[a]	34.4 ± 1.4[a]	36.0 ± 1.5[a]
Zn	343 ± 6.2[a]	341 ± 23[a]	349 ± 10.6[a]	355 ± 16.3[a]
Non-essential elements				
Ag	16.5 ± 0.8[a]	14.4 ± 1.5[a]	15.2 ± 1.7[a]	14.7 ± 1.1[a]
Al	<10	<10	<10	<10
Ba	0.4 ± 0.1[a]	0.5 ± 0.1[a]	0.4 ± 0.0[a]	0.5 ± 0.0[a]
Cd	0.3 ± 0.0[b]	0.3 ± 0.0[a,b]	0.4 ± 0.4[a]	0.4 ± 0.4[a]
Hg	0.1 ± 0.0[b]	0.2 ± 0.0[a]	<0.1	0.1 ± 0.0[a,b]
Pb	1.4 ± 0.0[b]	1.7 ± 0.0[a]	1.2 ± 0.0[c]	1.2 ± 0.1[c]

Elemental analyses were made from at least three replicates. Means ± SD with same superscript letters for the same row, denotes no statistical differences within the treatments ($P > 0.05$).
0.0 are values below 0.05.

Artemia and *Maja* were found in Cd, K, Mn, P and Zn content.

4. Discussion

4.1. Elemental content profiles of early stages of cephalopods

First investigations about trace elements in cephalopods have focused on essential elements, particularly on Cu because of its role in the haemocyanin (Ghiretti-Magaldi et al., 1958; Rocca, 1969; Nardi et al., 1971; Nardi and Steinberg, 1974) and to the interactions with non-essential elements (e.g., Martin and Flegal, 1975; Miramand and Guary, 1980; Smith et al., 1984; Finger and Smith, 1987; Miramand and Bentley, 1992). Most of these studies con-

cerned a single organ, mainly the digestive gland known to play a major role in the energetic metabolism of cephalopods, and also on the branchial hearts and their appendages which are involved in the excretion processes. These different works although limited to a narrow number of cephalopod species have clearly shown that these organs are deeply involved in the metabolism of Ag, Cd, Cu, Hg, ^{210}Po and Zn for the digestive gland and of ^{241}Am, Co, Fe, V and ^{237}Pu for the branchial hearts (Renzoni et al., 1973; Nardi and Steinberg, 1974; Martin and Flegal, 1975; Miramand and Guary, 1980; Guary et al., 1981; Smith et al., 1984; Finger and Smith, 1987; Miramand and Bentley, 1992; Bustamante et al., in press). Conversely, a limited number of studies have determined the concentrations in reproductive tissues as ovary and testis, and in the eggs (e.g. Bustamante et al., 1998b; Gerpe et al., 2000;

Craig and Overnell, 2003; Seixas et al., 2005; Miramand et al., 2006). These results were obtained with cephalopod collected from the field focusing on the levels of non-essential metals to infer their potentially toxic effects on the reproductive tissues and/or on the embryos. However, to the best of our knowledge the metabolic requirements of trace elements have not been studied to date in cephalopods.

The elemental composition of the octopus ovary and of the eggs, hatchlings and juveniles of the three cephalopod species analyzed here showed a high content in S. This high level can be expected because of the protein-rich, muscular body that characterizes cephalopods (Lee, 1994; Villanueva et al., 2004). For example, muscle S content reached up 75% of the total whole body burden of the purpleback flying squid *Sthenoteuthis oualaniensis* (Ichihashi et al., 2001a). The S concentrations recorded in eggs, hatchlings and juvenile of *S. officinalis*, *L. vulgaris* and *O. vulgaris* globally fall within the S concentrations reported for the muscle of the squid *Todarodes pacificus* (Median = 4700 μg g^{-1} wet weight) and in the ovary of the squid *S. oualaniensis* (3400 μg g^{-1} wet weight) (Ichihashi et al., 2001a,b). Sulphur is also abundant in the composition of hard structures of cephalopods such as the chitin of beaks (Hunt and Nixon, 1981), the

vestigial shell of adult *O. vulgaris* (Napoleao et al., 2005b), the gladius of adult *S. oualaniensis* (Ichihashi et al., 2001a), and the eggshell of the cirrate octopods (Villanueva, 1992).

The calcified shell of the cuttlefish originates the main structural and compositional difference among the species studied resulting in levels of Ca for *S. officinalis* more that five times that other hatchlings, in addition to remarkable high levels of Sr. In *S. officinalis*, the shell represents around 6% of the dry weight at hatching (Villanueva et al., 2004) and Ca and Sr are the most abundant elements in the calcified shell of adult cuttlefish *S. latimanus* (Ikeda et al., 1999) as well as important components of the ink of adult *S. officinalis* and *S. oualaniensis* (Sarzanini et al., 1992; Ichihashi et al., 2001a). The relatively high Ca and Sr content of the ink of cephalopods have been attributed to its richness in melanin (Sarzanini et al., 1992).

The embryonic development of nautiluses, cuttlefishes, squids and cirrate octopods occur in an eggs protected by a capsule which thickness varies according to the species. Inside the egg of cuttlefish, concentrations of non-essential elements (in ex. Ag, Cd, Pb) remains very low which suggest a limited transfer of these metals 1) towards the gonad during the maturation process and 2) through the eggshell during the embryonic development
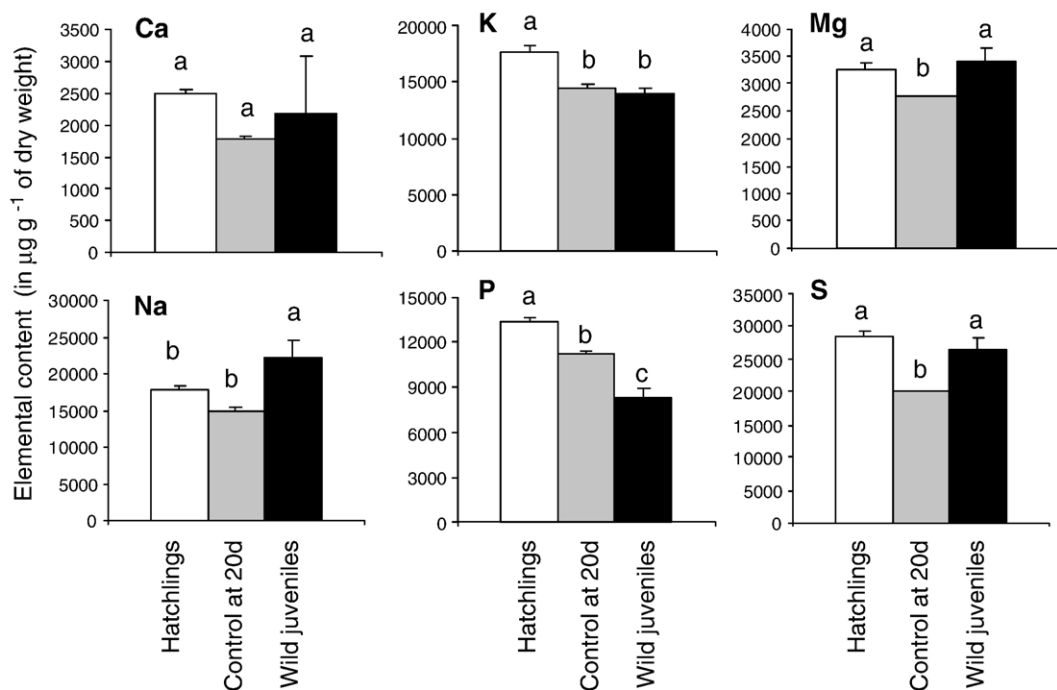

Fig. 1. Major essential elements in *Octopus vulgaris*. Comparison of the mean and standard deviation in content (μg g^{-1} dry weight, DW) of Ca, K, Mg, Na, P and S of hatchlings (mean DW 0.34 mg), reared individuals 20 days old from the control group feed with *Artemia* nauplii enriched with SuperSelco® (mean DW 0.68 mg), and wild juveniles (mean DW 1836 mg). Bars with the same letters are not significantly different ($P > 0.05$).

(Miramand and Bentley, 1992; Bustamante et al., 2002a, 2004; Miramand et al., 2006). In comparison, concentrations of essential trace elements are relatively elevated in the ovary, which is probably related to the high concentrations of metalloproteins such as Zn-rich proteins in this tissue (Gerpe et al., 2000). In the eggs, a very low percentage of Cu is under soluble form in Loliginid squids, suggesting the binding to a particular compound acting as a Cu reserve for the embryonic development (Craig and Overnell, 2003). Generally low coefficients of variation for both Cu and Zn in the eggs suggest a metabolic control of these elements. In *S. officinalis*, Cu and Zn concentrations in the hatchlings are close to those measured in the vitellus, probably constituting virtually the unique source of these elements for the embryos (Miramand et al., 2006).

By another hand, eggs of incirrate octopods (in ex., *O. vulgaris*) lacks eggshell and the chorion is in direct contact with the seawater. Present results showed that in comparison with recently spawned eggs, the developing *O. vulgaris* eggs have higher concentrations for most of the essential elements and also for some of the non-essentials (i.e., Ag and Pb). This difference with encased eggs could be due to the absorption of these elements from the seawater during the embryonic development in *O. vulgaris*.

Differences on the elemental composition during the ontogenetic development have been observed in cephalopods depending on the considered element and on the species (Bustamante, 1998). The content in non-essential elements found in the three species analyzed here seems to be lower in comparison with subadult and adult
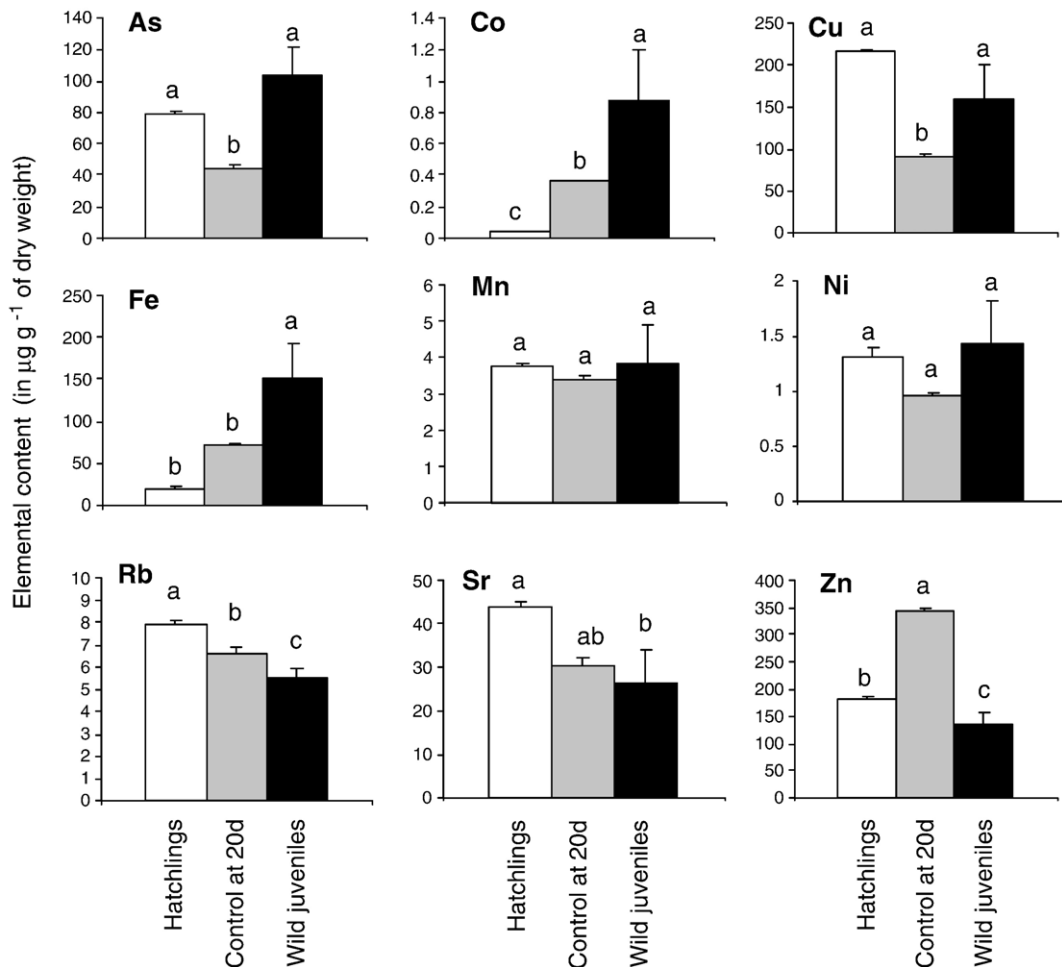

Fig. 2. Minor essential elements in *Octopus vulgaris*. Comparison of the mean and standard deviation in content of As, Co, Cu, Fe, Mn, Ni, Rb, Sr and Zn of hatchlings, reared individuals 20 days old and wild juveniles. Bars with the same letters are not significantly different (*P* > 0.05). Details as in Fig. 1.

stages of coastal cephalopods (Miramand and Guary, 1980; Miramand and Bentley, 1992; Seixas et al., 2005; Miramand et al., 2006). By another hand, levels of some essential elements are found in higher concentrations in the digestive gland of juvenile that adult squids *S. oualaniensis* for Ca, Cr, Na, Mg and Sr, and *Loligo forbesi* for Cd, which may attributable to diet ontogenetic changes (Ichihashi et al., 2001a; Stowasser et al., 2005).

4.2. Elemental requirements for early stages of cephalopods: an approach

Cephalopods are carnivorous, active predators and because they have very high feeding rates, most part of the elements can be assumed to be incorporated by the diet. However, absorption also takes place from seawater, as it occurs for instance for Ag (Bustamante et al., 2004; Miramand et al., 2006) and also probably for Hg (Bustamante et al., in press). In addition to an osmotic uptake through the gills and the body surface, in cephalopods seawater is taken into the gut by the mouth and rectal pumping and the digestive gland appendages are the principal site of fluid uptake, regulating ion balance (Wells and Wells, 1989). To the best of our knowledge, no data on the respective proportions of the

elements incorporated from food and seawater has been published to date for cephalopods. Fishes can take up significant amounts of Ca, K, Mg and Na from seawater (Lall, 2002); however, feed is the major source of essential elements such as Cu, Fe, Mn, P and Zn, which have low concentrations in seawater (Watanabe et al., 1997). A similar relationship can also be expected for cephalopods.

Present results for *S. officinalis* showed that Ca and Sr contents in wild juveniles increased more than three times the hatchling level, exceeding S concentrations. Seawater contains an appreciable amount of dissolved Ca that can be a source of this element for cephalopods. However, no studies on the Ca requirements have been done for this group of carnivorous molluscs. In other molluscs as in juvenile abalone, adequate Ca is probably obtained from the surrounding water and high levels of supplemental Ca did not significantly increase the tissue Ca content (Tan et al., 2001). In fish, the utilization of Ca from seawater varies according to the species. Calcium absorption from seawater can be sufficient to maintain stable the tissue Ca levels but do not provide enough Ca for normal fish growth. With a reduced Ca supply from the diet of some fish species, it results a significantly poorer growth and feed efficiency (Hossain and Furuichi, 2000). Under
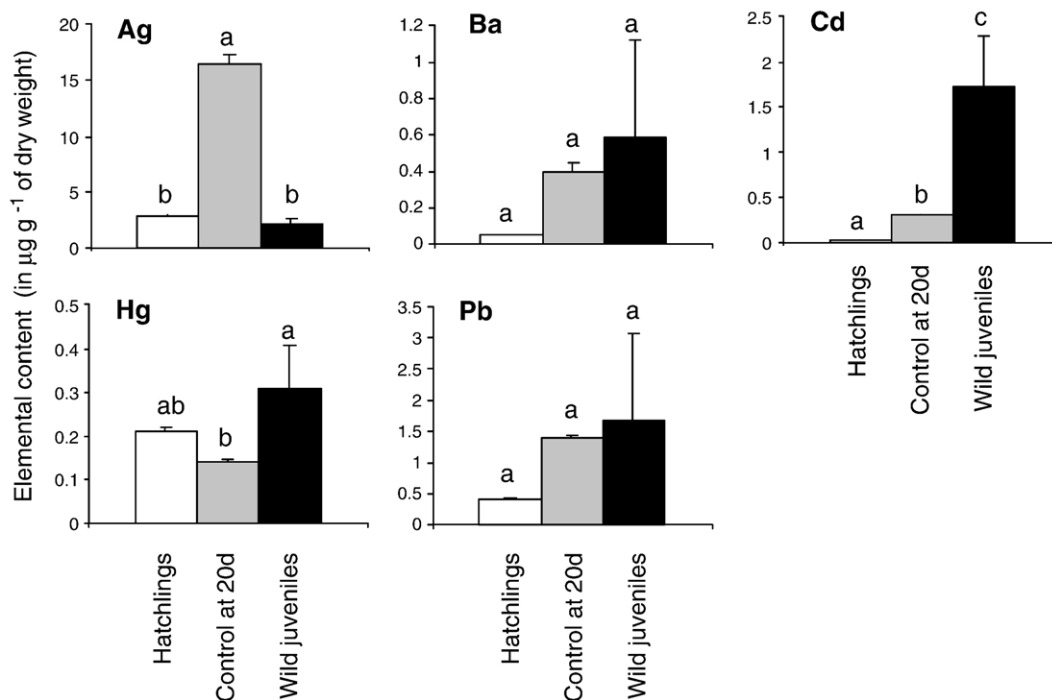

Fig. 3. Non-essential elements in *Octopus vulgaris*. Comparison of the mean and standard deviation in content of Ag, Ba, Cd, Hg and Pb of hatchlings, reared individuals 20 days old and wild juveniles. Bars with the same letters are not significantly different ($P > 0.05$). Details as in Fig. 1.

Table 5

Means±SD of the essential and non-essential elemental content (in μg g^{-1} of dry weight) of *Artemia* nauplii (used as food during the present study for cultures of *Octopus vulgaris* paralarvae) and hatchling decapod crab zoeae *Maja brachydactyla* (used as food for *O. vulgaris* paralarvae in previous studies)

	Artemia SS nauplii	*Artemia* MET nauplii	*Maja brachydactyla* zoeae
Major essential elements			
Ca	1105±63[b]	1020±62[b]	44533±2521[a]
K	14663±696[a]	14597±34[a]	15003±168[a]
Mg	2210±112[b]	1965±11[b]	10637±342[a]
Na	27301±1276[b]	27493±240[b]	51547±555[a]
P	12119±320[a]	12503±230[a]	12070±357[a]
S	7718±344[c]	10359±155[b]	13449±422[a]
Minor essential elements			
As	11.0±0.4[b]	11.4±0.1[b]	47.9±0.6[a]
Cr	<4	<4	<2
Co	0.4±0.0[a]	0.4±0.0[a]	<0.25
Cu	9.5±0.6[b]	7.9±0.0[b]	72.5±1.4[a]
Fe	100±5[a,b]	128±44[a]	44±2[b]
Mn	3.4±0.1[a]	3.3±0.1[a]	3.3±0.3[a]
Ni	6.0±0.3[a]	3.5±0.1[b]	<1
Rb	10.6±0.5[a]	10.5±0.1[a]	5.4±0.0[b]
Sr	14.7±1.0[b]	13.0±0.0[b]	564±35[a]
Zn	134±6.9[a]	136±1.3[a]	135±4.1[a]
Non-essential elements			
Ag	<0.14	<0.14	1.4±0.1
Al	<10	<10	<10
Ba	0.4±0.1[b]	0.3±0.0[b]	2.4±0.0[a]
Cd	0.1±0.0[a]	0.1±0.0[a]	0.1±0.0[a]
Hg	<0.14	<0.14	<0.14
Pb	0.5±00[b]	0.4±0.0[b]	0.6±0.1[a]

Artemia SS was enriched only with SuperSelco®, *Artemia* MET was enriched with SuperSelco® plus Methionine (see Materials and methods). Elemental analyses were made from at least three replicates. Means±SD with same superscript letters for the same element, denotes no statistical differences within the prey ($P>0.05$).
0.0 are values below 0.05.

experimental conditions, the shell growth rate of low-feeding, young cuttlefish *S. officinalis* strongly decreases with increasing starvation (Boletzky, 1974), indicating a possible feed requirement independent of a Ca intake from seawater. In the case of Sr, this element is incorporated into the squid and cuttlefish statolith from the diet (Hurley et al., 1985; Zumholz et al., in press) and from seawater (Hanlon et al., 1989). The calcified structures of cephalopods such as shell and statoliths require this element for normal embryonic development and survival. Egg incubations in artificial seawater without Sr produces abnormal hatchlings in the three species studied here and only normal development of the aragonite statoliths were obtained when Sr levels in seawater reach 8 mg l^{-1} (Hanlon et al., 1989).

An interesting element in cephalopods is Cu. This metal is required in large concentrations in cephalopods as it works as a respiratory pigment in hemocyanin which represents 98% of their blood proteins (Ghiretti, 1966; D'Aniello et al., 1986). From the literature, levels of Cu are reported for the gills, branchial hearts, digestive gland and muscle of adult cephalopods, including *O. vulgaris* (for recent literature review see Table 6 of Napoleao et al., 2005a). The Cu concentrations observed here in *O. vulgaris* hatchlings are similar to that reported for the gills of adults of the same species. The Cu abundance in the adult octopus gills may reflect the presence of haemocyanin, the dioxygen carrier Cu protein typical of molluscs and crustaceans (Taylor and Anstiss, 1999). However, these levels of Cu for the octopus hatchling, as a whole animal, seem to be relatively high compared to the adults. The richness in Cu of planktonic octopus may indicate a particular high nutritional requirement for this element. In decapod crustaceans, enzymatic requirements have been estimated to be around 26 μg g^{-1} of Cu and the total metabolic requirements (enzymes and haemocyanin) to be around 83 μg g^{-1} (Rainbow, 1988; Zauke and Petri, 1993). These estimated requirements for adult octopus are similar, reaching levels of 26 and 92 μg g^{-1} of Cu, respectively (White and Rainbow, 1985). Crustaceans constitute the main prey of many cephalopod species, particularly during paralarval and juvenile stages (Vecchione, 1991; Passarella and Hopkins, 1991) and crabs are the preferred prey of adult octopus in the wild (Nixon, 1987), between other reasons, probably because they are rich in Cu, Zn, cholesterol and n-3 fatty acids (King et al., 1990; Skonberg and Perkins, 2002). García García and Cerezo Valverde (2006) reported the optimal proportion of crabs in a fish +crab diet for ongrowing subadult *O. vulgaris*, noted that no cannibalism are reported when the minimum levels of crabs are maintained in the diet and pointed out a possible Cu dietary influence. In the same way, mortality associated with low Cu content diets has been also signalled for subadult cuttlefish *S. officinalis* (Castro et al., 1993).

The present results seems to confirm the importance of the Cu in the diet of *O. vulgaris* as 1) paralarvae of 20 d old feed on an *Artemia* diet showed significantly less Cu content that the "natural" Cu profile of hatchlings or wild juveniles, and 2) the paralarval group with poor survival (control) recorded the lower Cu content in comparison with the higher levels recorded for the group with best survival. In addition, prey composition analyzed here showed that *Artemia* nauplii have Cu levels 20 times lower that *O. vulgaris* hatchlings and 8 times lower that *M. brachydactyla* zoeae, a prey used previously with success as food for rearing *O. vulgaris* paralarvae (Carrasco et al., 2003; Iglesias et al., 2004). The low

levels in Cu of the reared *O. vulgaris* paralarvae feeding on *Artemia* nauplii may suggest that they are resulting from the low Cu content of *Artemia* and/or resulting from the poor physiological stage of the octopus paralarvae. Conclusions on this subject need further research, however, the Cu content of the *M. brachydactyla* zoeae (73 µg g^{-1}, see Table 5) may be considered as an optimal estimation of the Cu feeding requirement for *O. vulgaris* paralarvae under culture conditions. The deficient nutrient composition of *Artemia* as the sole larval food for *O. vulgaris* paralarvae is well known, particularly for lipid requirements (Navarro and Villanueva, 2000, 2003; Villanueva et al., 2002). However, in addition to other nutritional requirements, mainly from lipidic origin, Cu seems to be an important element on the paralarval octopus diet. Future studies are necessary to quantify these Cu dietary needs may testing a possible lipid+bioavailable Cu enriched *Artemia* suitable for paralarval octopus culture. To this respect, it should be borne in mind that Cu uptake by *Artemia* is influenced by pH and temperature (Blust et al., 1988, 1994).

In samples with low levels of Cu (fasted or reared paralarvae), Zn content increase notably compared to the hatchling or juvenile wild octopus Zn contents, that showed higher level of Cu. Similarly to Cd, Fe and Mo, Zn can act as a metabolic antagonist of Cu because of their similar nature of the valence shell hybrids and they compete for binding sites on proteins responsible for mineral absorption and/or synthesis of metalloenzymes (Watanabe et al., 1997; Lall, 2002). However, the details of such mechanisms in cephalopods are little known (Craig and Overnell, 2003). In adult octopus, enzymatic requirements have been estimated to be around 35 µg g^{-1} of Zn and the total metabolic requirements (enzymes and haemocyanin) to be around 81 µg g^{-1} (White and Rainbow, 1985). Zn is involved in numerous protein functions such as the carbonic anhydrase and is efficiently absorbed and strongly retained in *S. officinalis* both from the food and seawater pathways. In this species, the assimilation efficiency (AE) of Zn from food was higher for juveniles (AE=63%) than for adults (AE=41%) which may result from higher metabolic requirements in juveniles (Bustamante et al., 2002a,b).

In the reared *O. vulgaris* individuals, As, Mg and S also exhibited lower content in comparison with the "natural" profile of the hatchlings and wild juveniles (Figs. 1 and 2). In addition, Mg and S have lower contents on the reared groups with poor survival. The reduction of S concentrations may be due to a loss of muscle material and/or to the use of muscular protein to allow the organism at surviving. In fact, direct mobilization of muscle protein provides metabolic energy

during periods of starvation in adult octopus (O'Dor et al., 1984). Magnesium is an essential cofactor in many enzymatic reactions in intermediary metabolism, however, the Mg requirements for cephalopods are unknown. The high Mg content in seawater makes not necessary to supplement diets of seawater fishes as they obtain Mg by drinking (Lall, 2002) and a similar way can be expected for cephalopods. The content of As in the arms of adult *O. vulgaris* was supposed to be mainly under the non-toxic form, i.e., arsenobetaine (Seixas et al., 2005). The role of this element in early stages of cephalopods is also unknown.

5. Conclusion

Comparison of element concentrations in the eggs of cuttlefish, squid and octopus show that cephalopods may have developed different strategies regarding elemental requirements of the embryos with 1) eggs protected by an eggshell preventing from the incorporation of waterborne elements whatever they are essential or not 2) eggs with a chorion in direct contact with seawater, allowing the incorporation of various dissolved elements. However, in both cases, non-essential element concentrations remained globally low compared to juveniles or adults. On another hand, the present results on the elemental composition and both natural and artificial food strongly suggest that cephalopod paralarvae and juveniles must require a food rich in Cu. This is particularly clear for the octopus paralarvae and is probably related with the haemocyanin requirements for oxygen transport. In addition, the Ca requirements of the cuttlefish are also particularly high due to the well developed calcareous internal shell. At the present, the knowledge on the proportion of essential element incorporation from seawater or by food is lacking in this group of carnivorous molluscs and new research is urgently needed to understand the elemental requirements of cephalopods in culture.

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