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Vitamin A and E content in early stages of cephalopods and their dietary effects in *Octopus vulgaris* paralarvae

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ABSTRACT

The present study was designed to provide a look at the vitamin content of the early stages of cephalopods as an approach to their vitamin requirements in culture. Vitamin A and E profiles of the European cuttlefish *Sepia officinalis*, European squid *Loligo vulgaris* and common octopus *Octopus vulgaris* laboratory hatchlings and wild juveniles were analyzed. In addition, for *O. vulgaris* we determined vitamin A and E profiles of mature ovaries and eggs at different stages of development, and followed their possible dietary effects during the first month of paralarval rearing. We also analyzed vitamin A and E content of the live prey, i.e. *Artemia* nauplii, *Maja brachydactyla* hatchling crab zoeae and the mysidacean shrimp *Leptomysis buergii*. In the octopus ovaries and eggs, the vitamin A and E concentrations remained globally higher compared to paralarvae and wild juveniles. The vitamin A content in early stages of cephalopods was not much different from that observed in other marine molluscs and fish larvae and is expected to come from the carotenoid pool of their crustacean prey. Relatively high content of vitamin E was observed in the octopus ovaries, eggs, hatchlings and juveniles of the three cephalopod species analyzed. These levels are probably related to the high percentage of long chain polyunsaturated fatty acids (PUFA) that are particularly high in paralarval and juvenile cephalopods. The vitamin E content of the natural prey, *M. brachydactyla* and *L. buergii*, seemed to match or exceed the dietary needs of the three species of cephalopods analyzed. The vitamin E content of the *Artemia*-fed *O. vulgaris* increased during the rearing period and the content of the one month of age paralarvae did not differ from the content in wild octopus juveniles, suggesting that this prey may provide sufficient tocopherol for the young octopuses.

1. Introduction

Cephalopods are carnivorous, active predators and the shallow water species are characterized by rapid growth. Due to their rapid growth and market value, the culture of cephalopods is an area of increasing interest (Walsh et al., 2002; García García et al., 2004; Nabhitabhata et al., 2005; Sykes et al., 2006; Cerezo Valverde et al., 2008; Rosas et al., 2007, 2008). However, the rearing of the delicate early stages seems to be the main bottleneck for the development of the aquaculture of some species, such as *Sepia officinalis* (Domingues et al., 2001, 2003; Koueta et al., 2002; Koueta and Boucaud-Camou, 2003) and *Octopus vulgaris* (Itami et al., 1963; Villanueva, 1994, 1995; Iglesias et al., 2004, 2007; Okumura et al., 2005; Carrasco et al., 2006). Feeding the early stages of cephalopods, particularly of species with planktonic paralarval stages, is an unresolved problem and, at present, only cultures on an experimental scale using natural prey have been successful (Vidal et al., 2002; Villanueva and Norman, 2008). Aside from the problems related to food size and quantity, there seems to be other problems associated with food quality. Previous studies on the biochemical composition of the early stages of cephalopods and their feeding requirements have focussed on lipids (Navarro and Villanueva, 2000, 2003; Hamasaki and Takeuchi, 2001; Moxica et al., 2002; Domingues et al., 2004; Okumura et al., 2005; Almansa et al., 2006; Kurihara et al., 2006; Seixas et al., 2008), amino acids (Villanueva et al., 2004) and essential elements (Villanueva and Bustamante, 2006). These studies tried to design possible co-feeding techniques using *Artemia* and/or formulated diets suitable for paralarval and juvenile feeding (Villanueva et al., 1996, 2002; Domingues et al., 2001; Rosas et al., 2008). However, little information is available on the vitamin requirements of this group of molluscs and, as far as we know, no studies have been carried out relative to the vitamin content of the early stages of cephalopods. To test the potential benefit of dissolved nutrients on the survival of planktonic cephalopods, Forsythe and Toll (1991) studied the effect of vitamins on the planktonic stage of *Octopus joubini* by adding 1 mg l^{-1} of a vitamin complex to the rearing system every 3 days. They successfully reared the planktonic stage of *O. joubini* obtaining benthic juveniles, suggesting a possible positive effect of the waterborne vitamins.

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Among the vitamins, vitamins A and E play a key role in growth, reproduction and embryonic development. For example, it has been shown that the primary non-enzymatic antioxidants in fish eggs are vitamins E and A as well as provitamin A carotenoids. The content of these lipid-soluble vitamins in fish eggs has been suggested to result in larger hatchling size and larval survival (Lavens et al., 1999). In fact, vitamin A is a stimulus for new cell growth, aids in maintaining resistance to infection and is required for the regeneration of the two photo-pigments of the cephalopod retina: rhodopsin and retinochrome (Terakita et al., 1989; Blomhoff et al., 1992). Its importance in cephalopod vision has been discussed elsewhere (see among others, Fong et al., 1988; Saibil, 1990; Molina et al., 1992; Sweeney et al., 2007). Vitamin A is present in many forms including the free alcohol form retinol. Vitamin E is present in two structural types: as tocopherols and tocotrienols, and α-tocopherol has been the most studied form to date. However, recent studies have also shown the importance of γ-tocopherol in vertebrates (Wagner et al., 2004). Current information on the vitamin composition of cephalopods is limited to the subadult and adult forms in relation to their edible body portions (mantle and arms) or selected organs. The content of vitamins A and E in cephalopods has been reported by Fisher (1956), Sidwell et al. (1978), Motoe et al. (1997), Cho et al. (2001) and Passi et al. (2002). Less information exists for the water-soluble vitamins (Pandit and Magar, 1972; Sikorski and Kolodziejska, 1986).

The aim of the present study was to take a look at the vitamin content of the early stages of cephalopods in order to determine their requirements in culture. Therefore, we firstly determined the retinol and tocopherol composition of laboratory hatchlings and wild juveniles of three shallow water cephalopod species that represent the main cephalopod orders, all of which are of high commercial interest: the European cuttlefish *S. officinalis*, European squid *L. vulgaris* and common octopus, *O. vulgaris*. Secondly, for *O. vulgaris* we determined the same profiles in mature ovaries, eggs in different stages of development, hatchlings fasted for 4 days and reared paralarvae fed enriched *Artemia* nauplii. In addition, we analyzed cephalopod prey as hatchling zoeae of the spider crab *Maja brachydactyla*, a prey that has been successfully used as a food resource for rearing *O. vulgaris* during the planktonic stage (Iglesias et al., 2004; Carrasco et al., 2006) and the mysidacean shrimp *Leptomysis buergii*, because mysidacean shrimp have been used for rearing *L. vulgaris* paralarvae (Boletzky, 1979; Turk et al., 1986; Villanueva, 2000) and mysids has been cultured to feed *S. officinalis* hatchlings (Domingues et al., 1999, 2000, 2001).

2. Materials and methods

2.1. Collection of material

2.1.1. Cephalopod hatchlings and wild juveniles

Egg masses of *S. officinalis* and *Loligo vulgaris* were collected off Barcelona (NW Mediterranean) and egg masses of *O. vulgaris* were obtained from a broodstock maintained in the Institut de Ciències del Mar (ICM), Barcelona. Since the same specimens analyzed in the present study have been used for amino acid and elemental composition analysis, detailed information on the collection of material can be found in our previous studies (Villanueva et al., 2004; Villanueva and Bustamante, 2006). In short, healthy individuals of the 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 vitamin analysis.

To determine retinol and tocopherol in wild juveniles, 5 *S. officinalis* juveniles (25.8–30.7 g wet weight) collected from the artisanal fishery off Barcelona; 5 *L. vulgaris* juveniles (1.7–7.4 g wet weight) collected from the local trawl fishery off Tarragona (NW Mediterranean) and 4 benthic *O. vulgaris* juveniles (9.2–14.2 g wet weight) captured from the wild by scuba diving off L'Estartit (NW Mediterranean) were analyzed (Table 1). All juveniles were weighed fresh upon arrival in the laboratory and then frozen at −80 °C.

2.1.2. Rearing experiments of O. vulgaris paralarvae

Specimens analyzed here were from a culture experiment reported by Villanueva et al. (2004) as culture METAA, where detailed information on rearing methods can be found. In short, paralarvae were reared for 30 days using cylindrical 25-l volume PVC tanks at mean temperature of 20.4 °C (range 19.2–21.1 °C). The culture experiment was conducted in quadruplicate. Paralarvae were fed enriched *Artemia* nauplii (AF, INVE Aquaculture) 450 μm in length, which were provided from day 0 to day 20 at a ratio of 6–7 nauplii ml^{-1} d^{-1}, decreasing to 4 nauplii ml^{-1} d^{-1} thereafter until an age of 30 days, due to a decrease in paralarval survival and density (see Villanueva et al., 2004 for details). *Artemia* nauplii were enriched in seawater for 24 h at 28 °C with 0.6 g l^{-1} of DC Super Selco (INVE) 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 were added to the rearing tanks (see Villanueva et al., 2004 for details). Paralarval samples were collected 2 h after the first daily feeding, 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 vitamin analysis. An unfed group was also maintained from the hatchling stage to day 4.

2.1.3. Paralarval prey

The retinol and tocopherol content of *Artemia* nauplii were analyzed because this prey was used as food during the present study (identified as Artemia MET in Villanueva et al., 2004). In addition, the retinol and tocopherol content of recently hatched

Table 1
Means±SD of the wet and dry weights (mg ind^{-1}) of *S. officinalis*, *L. vulgaris*, and *O. vulgaris* hatchlings and wild juveniles in relation to their content of retinol and tocopherol (μg g^{-1} dry weight)

	Hatchlings	Wild juveniles	
	Mean±SD	Mean±SD	Range
S. officinalis			
Wet weight	87.8±5.7	31,890.0±5669.0	25,820–39,700
Dry weight	22.7±1.6	8517.6±1641.2	7164–11,143
Retinol	0.6±0.2[b]	2.6±0.2[a]	
Tocopherol total	62.3±22.3[b]	483.3±132.7[a]	
α-Tocopherol	62.3±22.3[b]	311.5±85.9[a]	
γ-Tocopherol	0.0±0.0[b]	171.7±46.8[a]	
L. vulgaris			
Wet weight	3.8±0.1	3652.8±2250.8	1723–7437
Dry weight	0.9±0.0	813.6±506.9	361–1645
Retinol	0.9±0.1[b]	7.1±0.7[a]	
Tocopherol total	361.2±13.5[b]	615.0±100.5[a]	
α-Tocopherol	199.1±11.5[b]	317.6±45.8[a]	
γ-Tocopherol	162.1±4.6[b]	297.4±64.4[a]	
O. vulgaris			
Wet weight	2.1±0.0	11283.5±2082.1	9240–14,188
Dry weight	0.3±0.1	2990.0±973.5	1419–3671
Retinol	1.6±0.3[a]	2.2±0.8[a]	
Tocopherol total	691.6±69.2[a]	343.3±59.6[b]	
α-Tocopherol	424.5±21.2[a]	343.3±59.6[b]	
γ-Tocopherol	267.1±55.4[a]	ND	

0.0 are values below 0.05.
Means±SD with the same superscript in the same row denotes no statistical difference between hatchlings and wild juveniles ($P>0.05$). ND, not detected.