Chapter 5
Nutrition as a Key Factor for Cephalopod Aquaculture

Juan Carlos Navarro, Óscar Monroig and António V. Sykes

Abstract Cephalopods are fast-growing animals, active swimmers and top preda-
tors, which require substantial amounts of food. As such, they show high metabolic
rates dependent on a carnivorous diet, thus hypothetically linked to a predominant
amino acid metabolism. Their body composition is mainly constituted by high lev-
els of total protein, and their lipids, although quantitatively low, reveal the presence
of substantial amounts of long-chain polyunsaturated fatty acids. All in all, little is
known about their nutritional requirements, especially during the early stages, very
prone to high mortalities under culture. This chapter is a brief account of key infor-
mation concerning relevant points linked to the nutritional requirements that cepha-
lopods have for proteins, lipids, carotenoids, carbohydrates, minerals and vitamins.
Moreover, some considerations on populational metabolism are also presented.

Keywords Amino acids · Carbohydrates · Fatty acids · Lipids · Nutrition · *Octopus
vulgaris* · Populational metabolism · Proteins · *Sepia officinalis*

5.1 Introduction

Cephalopods potential for aquaculture production was acknowledged by several
researchers in the last decades of the twentieth century (e.g. Hanlon 1987; Bou-
caud-Camou 1989, 1990; Hanlon et al. 1991; Barnabé 1996). Nonetheless, several

J. C. Navarro (✉) · Ó. Monroig
Instituto de Acuicultura Torre de la Sal (IATS-CSIC), 12595 Ribera de Cabanes,
Castellón, Spain
e-mail: jcnavarro@iats.csic.es

Ó. Monroig
Institute of Aquaculture, School of Natural Sciences, University of Stirling,
Stirling FK9 4LA, Scotland, UK
e-mail: oscar.monroig@stir.ac.uk

A. V. Sykes
CCMAR-CIMAR L.A., Centro de Ciências do Mar do Algarve, Universidade do Algarve,
Campus de Gambelas, 8005-139, Faro, Portugal.
e-mail: asykes@ualg.pt

bottlenecks remain and are impeding the transition of technology from pilot to full-scale, namely the existing knowledge on cephalopod nutrition. Nutrition is a key factor for proper growth and survival under captive conditions and mass culture. This chapter picks up from the last review on this subject, made by Lee (1994). Firstly, it presents a nutritional approach, which is mostly based on the biochemical composition of both cephalopods and preys, and finalizes with a metabolic hypothesis, which considers other variables such as enzymes, geographical adaptation and stress. Due to the low amount of existing information for most cephalopod species, this chapter mainly focuses on nutritional studies conducted in *Sepia officinalis* and *Octopus vulgaris*, arguably the cephalopod species of greatest commercial interests.

5.2   Proteins

Proteins are the most abundant macronutrient in cephalopods (Zlatanos et al. 2006) and, as stated by Lee (1994), large protein and amino acid contents in the diet of cephalopods are required for sustaining growth and fulfilling energy demands. According to Lee (1994), cephalopods efficiently absorb, digest and utilize dietary proteins that are further used for locomotion, structural support, energy source, oxygen transport and osmoregulation. Cephalopods display high rates of protein synthesis and retention, and low rates of protein degradation (Houlihan et al. 1990; Moltschaniwskyj and Carter 2010). In order to evaluate the dietary requirements of the common octopus *O. vulgaris* during the fast-growing stages, the total and free amino acid (FAA) composition of paralarvae and juveniles was determined by Villanueva et al. (2004). Similar to the amino acid content found in the mantle of juveniles of *O. vulgaris*, *S. officinalis* and *Loligo vulgaris* by Zlatanos et al. (2006), these authors found that glutamate and aspartate were the most abundant nonessential amino acids (NEAAs) in *O. vulgaris* paralarvae, with lysine, leucine and arginine accounting for nearly half of the essential amino acids (EAAs). Interestingly, arginine was the most abundant FAA in paralarvae, possibly indicating its further use for octopine formation produced during an anaerobic work (Baldwin et al. 1976; Hochachka et al. 1976, 1983; Storey and Storey 1979; Storey et al. 1979; Hochachka and Fields 1982) or an active metabolism for energy production and biosynthesis of other amino acids. On the other hand, cephalopods might use proline during oxidative metabolism, either as energy source or as means for augmenting the Krebs cycle (Hochachka and Fields 1982). Both arginine and proline are potentially interconvertible through glutamate and ornithine (Mommsen et al. 1982).

In a general way, there is already information on the amino acids of *O. vulgaris* prey species, such as for *Artemia* spp. (Aragão et al. 2004) and crustaceans such as the spider crab (Andrés et al. 2010), as well as for raw materials used for prepared diets (Valverde et al. 2013). Cephalopod's growth is primarily an increase in body muscle mass by protein synthesis and accretion, and individuals display very high growth rates (especially at the paralarvae and hatchling stages), which means that they have a high dietary requirement for amino acids. In addition, these high growth

rates are most probably due to highly efficient ingestion, digestion (Boucher-Rodoni et al. 1987) and assimilation of protein (Domingues et al. 2005), which have to be supplied by a diet with balanced levels of amino acids, despite the capacity of some cephalopods to perform integumental amino acid uptake from seawater (de Eguileor et al. 2000; Villanueva et al. 2004). Recent results point to the possibility that cuttlefish might not be able to use protein that has been denatured (Domingues et al. 2009) and to the favour on amino acid use through the pyruvate and tricarboxylic acid pathways in detriment of the ketogenic pathway in starving *O. vulgaris* (García-Garrido et al. 2012). Given its importance for cephalopods metabolism (Lee 1994), there is a need for characterization of the amino acids pool (these should be predominantly used as metabolic fuel, but they should also be utilized for body protein synthesis) at different life stages of different species and preys used for cephalopod rearing. The latter assumes greater importance since marine fish larvae seem to have a lower capacity to digest and absorb complex proteins than juvenile fish (Conceição et al. 2010), and display high amino acid requirements for protein deposition, turnover and catabolism to attain rapid growth (Rønnestad et al. 2003).

In a similar way to demersal fish eggs (Rønnestad et al. 1999), the FAA and protein amino acid pools of *S. officinalis* wild and culture eggs are largely dominated by taurine (more than 50 %; Sykes et al. unpublished data). Taurine is an amino acid analogue that is not incorporated into protein, but well known for its multiple roles that include inotropy of high and low calcium, modulator of neuron excitability, resistance to anoxia and hypoxia, bile salt synthesis and simulation of glycolysis and glycogenesis (Huxtable 1992). Taurine is also abundant during planktonic stages of the common octopus where it might play a role in osmoregulation (Villanueva et al. 2004). Nonetheless, its effect on metabolism and growth performance in a fish like gilthead seabream was only related to the increasing methionine availability for several important physiological purposes (Pinto et al. 2013).

5.3 Lipids

Little is known about the lipid requirements of cephalopods, apart from the general information drawn from the analysis of their body composition and assumptions made from their feeding habits. It is then difficult to separate early stages from adult requirements, or establish specific differences. Efforts devoted to the culture of certain species like *O. vulgaris* and *S. officinalis* have provided some information.

Very low levels of total lipids (TL) are present in the mantle of adults (Boucaud-Camou 1990; Sykes et al. 2009b) and hatchlings (Navarro and Villanueva 2000, 2003). This fact, combined with their poor capacity for mitochondrial lipid oxidation (O'Dor et al. 1984; Hochachka 1994), has somehow put aside research on cephalopod lipid nutrition until recently, when research on the causes of the massive mortalities encountered during the culture of early stages of merobenthic species has brought back the protagonism of these essential components on cephalopod nutrition, perhaps with an overemphasis on quantitative rather than qualitative points of view, given the poor essential lipid composition of the live preys commonly used

in aquaculture. Besides, other particular aspects, like the lipid-rich nervous system of hatchlings of *O. vulgaris* paralarvae representing approximately one quarter of the animal's fresh weight (Packard and Albergoni 1970), suggest the importance of lipids for suitable growth during planktonic life. Navarro and Villanueva (2000, 2003) made the first approach towards the study of lipid requirements in early stages of cephalopods to conclude that a nutritional imbalance in the lipid and fatty acid (FA) profile of the artificial feeding protocol may be responsible for the high mortalities encountered. In particular, *O. vulgaris* should require feeding on low-lipid preys, rich in polar lipids (PL), long-chain polyunsaturated fatty acids (PUFA) and possibly cholesterol (Navarro and Villanueva 2000, 2003; Okumura et al. 2005; Seixas et al. 2008). This closely resembles the composition of a 'natural' diet based on crustacean larvae and other marine planktonic organisms like copepods, but is far from the typical composition of the enriched *Artemia* spp. in any of its forms. The picture is even more complicated after evidences pointing at the paralarvae as specialist predators, contrary to the general concept by which they had been often regarded as generalist predators (Roura et al. 2012).

On the other hand, cuttlefish are unable to store lipids in the digestive gland (Fluckiger et al. 2008) and require high levels of phosphatidylcholine, phosphatidylethanolamine (PE) and cholesterol in their diets (Almansa et al. 2006). This conclusion was drawn by the latter authors based on data of whole animal (Navarro and Villanueva 2000), cuttlefish mantle (Sinanoglou and Miniadis-Meimaroglou 1998, 2000) and also of prey lipid content (Domingues et al. 2003; Domingues et al. 2004). It is, however, interesting to verify that these same lipid classes have the highest levels throughout cuttlefish wild and culture egg embryonic development and that, although there is a difference in the amount of TL of eggs from different geographical locations (Sykes et al. 2009a), there is maintenance on the amount of TL until hatching (Bouchaud and Galois 1990). According to Bouchaud and Galois (1990), the egg-yolk lipids correspond to 14 % dry weight in eggs and 15 % in hatchlings (mainly phospholipids that may be used for energetic purposes).

However, in the case of octopus, up to now, there is no paralarval food from the aquaculture artificial food chain that can compare with the lipid composition of natural live food, and every effort has to be made to try to increase the essential long-chain PUFA and PL content of live preys. In fact, it is not only the bulk provision of essential lipids that is important but also the adequate lipid form (Guinot et al. 2013). This scenario may be more important for early stages, and even paramount in some animal groups like cephalopods, whose FA composition is essentially constituted by palmitic acid (16:0), stearic acid (18:0), docosahexaenoic acid (22:6n-3, DHA) and eicosapentaenoic acid (20:5n-3, EPA), the latter two being essential long-chain highly unsaturated fatty acids (HUFA) for marine organisms (Sinanoglou and Miniadis-Meimaroglou 1998; Navarro and Villanueva 2000; Passi et al. 2002; Ozyurt et al. 2006; Zlatanos et al. 2006). However, the most recent results from Seixas et al. (2010) do not completely exclude the importance of DHA for the successful rearing of *O. vulgaris* paralarvae but seem to point that maybe other n-3 HUFA would be more vital. Very interesting results from Quintana (2009) have shown that EPA is particularly important in paralarvae lipids, with 1:1 EPA:DHA proportions

in the PE, as opposed to the 1:2 proportion generally reported for marine fish. Likewise, EPA is higher in shrimp than fish diets tested as cuttlefish food (Domingues et al. 2003) and its importance for normal growth was proposed by Almansa et al. (2006). The latter authors also indicated that cuttlefish metabolism of both EPA and DHA might be somehow different from fish, where in general the reduction of EPA and DHA sources implies a reduction of the level of these FA in several tissues (Sargent et al. 1995). In addition, Domingues et al. (2003) indicated a 2:1 EPA:DHA proportion in the prey supplied to hatchlings and, in the Ferreira et al. (2010) work, cuttlefish juveniles attaining the best growth and survival rates were fed shrimp with a similar EPA:DHA ratio and PUFA content. On the other hand, Koueta et al. (2002) and Perrin et al. (2004) have suggested the importance of these FAs and PUFA in cuttlefish hatchling survival when facing a stressful situation (either lower water quality or improper prey size, respectively). This suggests the importance of PUFA in cephalopod nutrition and may suggest, for example, that in addition to DHA, EPA may play an important role in the brain and visual system of cephalopods.

A closer look at the available results on the FA profile of *O. vulgaris* paralarvae (Navarro and Villanueva 2000; Miliou et al. 2006) reveals that, apart from the high levels of DHA, 16:0 and EPA, arachidonic acid (20:4n-6, ARA) is one of the most abundant FA, with surprisingly high values of 18:2n-6 and other n-6 FA being found in marine species. From these and other findings in other marine molluscs (Uki et al. 1986; Dunstan et al. 1996; Durazo-Beltran et al. 2003), it has been suggested that 20:4n-6 might not be essential, since the ability for enzymatic bioconversion from adequate precursors to 20:4n-6 could be present in this species. On the other hand, 18:3n-3 is not present in octopus paralarval tissues (Navarro and Villanueva 2000, 2003; Miliou et al. 2006) although this FA is massively included through the *Artemia* spp. feeding. Moreover, 18:3n-3 may compete with 24:5n-3 for the Δ6 desaturase in the metabolic route leading to DHA production. Therefore, it might be possible that a diet rich in 18:3n-3 could be affecting the proper production of n-3 essential FA (EFA) of paralarvae. An opposite hypothesis is that the C18 desaturation–elongation pathways of 18:3n-3 and 18:2n-6 may be active in *O. vulgaris* so as to produce physiologically essential n-3 and n-6 FA. To explore these and other aspects of lipid metabolism research on dynamic aspects of lipid nutrition and metabolism by characterizing enzymes involved in lipid biosynthesis in these organisms, i.e. desaturases and elongases, is an invaluable tool. Monroig et al. (2012a) have recently isolated a complementary DNA (cDNA) with high homology to fatty acyl desaturases (Fad) in adult octopus. Functional characterization of this enzyme showed that the octopus Fad exhibited Δ5-desaturation activity towards saturated and polyunsaturated fatty acyl substrates. Thus, it efficiently converted 16:0 and 18:0 to 16:1n-11 and 18:1n-13, respectively, and desaturated PUFA substrates 20:4n-3 and 20:3n-6 to 20:5n-3 (EPA) and 20:4n-6 (ARA), respectively. Although the Δ5 Fad enables common octopus to produce EPA and ARA, the low availability of its adequate substrates 20:4n-3 and 20:3n-6, either in the diet or by limited endogenous synthesis from C18 PUFA, might indicate that EPA and ARA are indeed EFA for this species. Interestingly, the octopus Δ5 Fad can also participate in the biosynthesis of non-methylene-interrupted diene (NMID) FA, PUFA that

are generally uncommon in vertebrates but have been found previously in marine invertebrates, including molluscs, and now also confirmed to be present in specific tissues of common octopus.

Molecular cloning and functional characterization of a cDNA encoding a putative elongase of very long-chain fatty acids (Elovl), a critical enzyme that catalyzes the elongation of FA including PUFA, in common octopus, suggests its phylogenetic relation to Elovl5 and Elovl2, two elongases with demonstrated roles in PUFA biosynthesis in vertebrates (Monroig et al. 2012b). Functional characterization of the octopus Elovl showed the ability to elongate some C18 and C20 PUFAs, while C22 PUFA substrates remained unmodified. Interestingly, the octopus Elovl *elongates* n-6 PUFA substrates more efficiently than their homologous n-3 substrates, suggesting that n-6 PUFA may have particular biological significance in *O. vulgaris,* as mentioned above, and stressing again the essentiality of long-chain n-3 PUFA, and in particular DHA. Besides, the elongase also plays a pivotal role in the biosynthesis of NMID FA.

Similar studies carried out in other cephalopods like *S. officinalis* show a strict parallelism in terms of both qualitative FA composition (see Navarro and Villanueva 2000; Almansa et al. 2006) and biosynthetic capacity (Monroig et al., unpublished data). This, along with tissue FA composition (Monroig et al. 2012a), emphasizes the importance and essentiality of long-chain FA for cephalopods, and validates the idea of using the information to establish the guidelines of the requirements for coastal cephalopods. The recent manuscript by Valverde et al. (2012) on lipid classes from marine species and meals intended for cephalopod feeding has provided additional information on lipids.

5.4   Carotenoids

Carotenoid deposition has been described in cephalopods at the digestive gland (Fox 1966) and in accessory nidamental glands (Decleir and Richard 1972; Van den Branden et al. 1978; Van Den Branden et al. 1980) of *S. officinalis* and proposed to be designated as sepiaxanthine, but there is scarce information about its physiological role. Carotenoid astaxanthin can also be deposited in the skin of *S. officinalis* when fed on grass shrimp (*Palaemonetes varians*; Almansa et al. 2006) and this prey, used for cuttlefish hatchlings rearing, displays up to ten times more carotenoid content (Domingues et al. 2004). *O. vulgaris* paralarvae seem to be able to deposit part of the canthaxanthin present in *Artemia* spp. and to metabolize this carotenoid to astaxanthin (Rodríguez et al., Universidad de La Laguna, unpublished data). Fisher et al. (1956) reported the existence of vitamin A and, in some species, β-carotene in cephalopods. Taking into account the pro-vitamin A and antioxidant activity of carotenoids (Liñán-Cabello et al. 2002), and the importance of this vitamin in photoreception, growth and development, it would be very interesting to carry out more studies to determine the role of carotenoids in early stages of cephalopods as suggested by Villanueva et al. (2009).

5.5 Carbohydrates

Contrary to proteins and lipids, carbohydrate (CH) nutrition in the common octopus has barely been investigated. Considering their limited abundance compared to other macronutrients (normally under 1 % of total dry weight; Vlieg 1984; Kreuzer 1984), it is generally accepted that cephalopods do not have a specific requirement for dietary CH (Lee 1994). While protein and amino acids are the primary energy source for cephalopods, it has been reported that cephalopods including *O. vulgaris* are able to rapidly catabolize dietary CH to account for energy demands in explosive activities such as prey capture and fleeing from predators (Morillo-Velarde et al. 2011). Therefore, CH may significantly contribute to fuelling metabolism in *O. vulgaris* under starvation conditions and, consequently, adequate CH inclusion of diets for octopus culture should not be underestimated. The source of CH utilized in diet formulation also needs to be considered. For instance, while glucose was easily digested by *O. vulgaris* (O'Dor et al. 1984), a recent study has revealed that other sugar types such as starch present in freeze-dried pea may exhibit extremely low digestibility (Morillo-Velarde et al. 2012).

On the other hand, the existence of a CH metabolism has been suggested by Sykes et al. (2009a), due to different temperatures of specific geographical locations that may influence the egg nutritional content and metabolism in *S. officinalis*. The sepia egg yolk is composed by a water-soluble glyco-lipoprotein (Ito et al. 1962; Blanchier 1981). This glyco-lipoprotein has 20 % lipids (with 65 % phospholipid and minor or no cholesterol contents) and 12.6 % of CHs (Ito and Fujii 1962). In fact, cuttlefish eggs from Faro (Portugal) have more CHs than lipids (Sykes et al. 2012). Bouchaud (1991) studied the energetic expenditure of *S. officinalis* during embryonic development and found that eggs with more than 0.075 g displayed a similar amount of energy (1,600 J), which led to a theory on the use of yolk for growth and catabolic purposes that is inversely correlated with temperature (e.g. higher temperature implies higher catabolism). In addition, higher temperatures will imply higher oxygen uptake by the embryo (Wolf et al. 1985), which is attained by the increased water volumes of eggs (Sykes et al. 2009a).

5.6 Minerals

The elemental requirements of *O. vulgaris*, as for cephalopods in general, are poorly understood. Nevertheless, it is accepted that octopuses, as carnivorous species, meet the majority of their elemental requirements from the diet, although direct uptake from the seawater has also been shown to occur through an ion balance mechanism regulated by the digestive gland appendages (Wells and Wells 1989). A literature review on the element concentrations in a series of tissues from cephalopods was reported by Napoleão et al. (2005a). While specific (quantitative) requirements for both essential and nonessential elements have not yet been determined, some

studies aiming to determine the elemental composition of *O. vulgaris* have been conducted as a first approach to establish the dietary requirements in this species (Napoleão et al. 2005a; Napoleão et al. 2005b). Thus, Villanueva and Bustamante (2006) reported the elemental composition of the mature ovary, hatchlings, eggs at different developmental stages, wild juvenile individuals and also paralarvae fed a variety of experimental diets. Generally, S, Na, K, P and Mg were determined as the most abundant elements in *O. vulgaris*. Compared to other cephalopods, hatchlings from *O. vulgaris* contained higher levels of Ag, Cu, Mn, Ni and Zn. Compared to subadults and adults of the common octopus (Seixas et al. 2005), the contents of some nonessential elements, namely Ag, Al, Ba, Cd, Hg and Pb, were lower in hatchlings and reared paralarvae, suggesting an accumulation of such elements during development. Similar accumulation of oligoelements seems to occur in cuttlefish (Lacoue-Labarthe et al. 2008a, b, 2009, 2010a, b, Lourenço et al. 2009). Certain elements with potentially pivotal roles in the octopus and cuttlefish physiology have been studied more extensively. Copper (Cu), a key element in the respiratory function of haemocyanin (Ghiretti 1966; D´Aniello et al. 1986), has been postulated to be required for octopus paralarvae, as suggested by the high levels encountered in paralarvae fed on the natural prey *Maja brachydactyla* zoeae in comparison with *Artemia* spp. nauplii (Villanueva and Bustamante 2006). Moreover, octopuses fed on crustacean-based diets contained increased Cu levels compared to fish-fed octopuses, and therefore hypothesized to partly account for a higher cannibalism rate in the latter (García-García and Cerezo-Valverde 2006). The importance of Cu is also high in *S. officinalis* (Decleir et al. 1978), especially during maturation of haemocyanin (Decleir and Richard 1970; Decleir et al. 1971; Wolf et al. 1980; Beuerlein et al. 2004), and this probably extends to other cephalopods species (Taylor and Antiss 1999). Similar to octopus, the low content of Cu in prepared diets has been pinpointed to provoke mortality by Castro et al. (1993).

Sulphur (S) is also regarded as an essential element for the common octopus and cuttlefish that needs to be provided in the diet at high quantities to sustain formation of muscular proteins (Lee 1994; Villanueva et al. 2004), vestigial shell (Napoleão et al. 2005b) and chitinized structures such as beaks (Hunt and Nixon 1981). Other elements such as strontium (Sr) and cobalt (Co) appear to be incorporated by the common octopus directly from the seawater and by food intake in cuttlefish. It has been demonstrated that Sr is critical for adequate statolith development and consequently for normal swimming and survival of newly hatched octopus, among other cephalopods (Hanlon et al. 1989). In addition to its potential role as an integral component of vitamin B_{12}, Co has also been pointed out as important in the development of adenochrome, a pigment found in the branchial heart, and therefore with a potential role in excretion (Miyazaki et al. 2001).

The cuttlefish *S. officinalis* has a cuttlebone which is made of calcium aragonite (Hewitt 1975) and may suffer malformation resulting from malnutrition (Boletzky 1974). Since food is the primary pathway for the accumulation of trace elements (Bustamante et al. 2004), the inclusion of calcium in a prepared diet is implicit. In fact, the mineral fraction of cuttlefish accounts for 22–32% in eggs (Sykes et al. 2012) and 6% of dry weight at hatching (Villanueva et al. 2004), which can only be attained in such way.

5.7 Vitamins

Villanueva et al. (2009) reported that information on the vitamin composition of cephalopods is mainly limited to the subadult and adult forms in relation to their edible body portions (mantle and arms) or selected organs (Fisher 1956; Sidwell et al. 1978; Motoe et al. 1997; Cho et al. 2001; Pandit and Magar 1972; Passi et al. 2002; Sikorski and Kolodziejska 1986). These authors analysed the vitamin content of the early stages of cephalopods as an approach to establish their requirements in culture. Antioxidants such as tocopherols are regarded to be very important for the prevention of lipid oxidation, particularly α-tocopherol since it is degraded to protect PUFA against oxidation in fish larvae (Sargent et al. 1997). In fact, it has been reported that the α-tocopherol requirement may depend on the dietary PUFA level (Stéphan et al. 1995; Halver 2002; Brown et al. 2005). Vitamin A and E (α- and γ-tocopherols) profiles of the European cuttlefish *S. officinalis,* European squid *L. vulgaris* and common octopus *O. vulgaris* laboratory hatchlings and wild juveniles were determined. The vitamin A content in early stages of cephalopods was not much different from that observed in other marine molluscs and fish larvae. Besides, relatively high content of vitamin E was observed in the hatchlings and juveniles. These authors postulated that the high levels of vitamin E are probably associated with the high percentage of oxidation-prone PUFA that are particularly high in paralarval and juvenile cephalopods. In general terms, they concluded that the natural and artificial preys (*Artemia* spp.) of early stages of cephalopods either fulfilled their vitamin requirements directly or provided precursors (i.e. carotenoids) that could be transformed in vitamins.

5.8 Populational Metabolism Differences

Captive cephalopods have a different physiology than those from the wild and their tissues are characterized by thicker mantles, a greater proportion of mitochondria-rich tissue, muscle fibres with smaller mitochondrial cores and fewer small muscle fibres (Pecl and Moltschaniwskyj 1999). This suggests a reduced rate of new fibre generation, indicating an alteration to the cellular growth mechanisms and not simply a change in the physiological growth rate observed in several laboratory and field studies (Semmens et al. 2004). Growth registered in captive individuals is usually lower than that estimated or verified for those from nature (an example of this is presented in Chap. 11, in the part of cuttlefish culture in earthen ponds).

As stated by Sykes et al. (2006), the particular metabolism of cephalopods needs to be considered if a successful artificial diet is to be designed. The partitioning of the dietary energy intake by cephalopods in the various types of metabolism, excretion and growth was reviewed by O'Dor and Wells (1987). Bearing that in mind, the nutritional content of the diet must be good enough to sustain the existing cephalopod metabolic costs, so the animal is able to allocate an optimal distribution of surplus

energy to somatic growth and, later, to reproduction (Wells and Clarke 1996). There-fore, the animal has to feed on a diet correctly balanced to its metabolic needs at a given temperature (André et al. 2009). The existing information is resumed to two theories that clash in terms of what the cephalopods use as energy substrate. The first one, by Lee (1994) and Boucher-Rodoni and Mangold (1994), considers that under normal feeding conditions, both growth and energy use the protein fraction as fuel. The second theory, by Storey and Storey (1983) and Hochachka (1994), considers that the CH fraction is used as energy source and the protein fraction is exclusively used for growth. Such contrasting theories may originate from the fact that the same species is physiologically adapted to a given geographical location, with different temperature regimes and food nutritional composition. For instance, *S. officinalis* populations from the English Channel and southern Portugal have been reported to be genetically different (Wolfram et al. 2006). In addition, this species displays a physiological plasticity (Oellermann et al. 2012), which is temperature- and food-dependent (reliant on the lifestyle of cephalopods and their low energy reserves).

Cephalopods have appropriate catabolic pathways to breakdown protein to amino acids to obtain energy (Ballantyne et al. 1981). However, most cephalopods' living strategy is to lay down protein reserves into rapid growth to convert them into gam-etes (O'Dor et al. 1984; Moltschaniwskyj and Carter 2013). Hypothetically, it would be a waste to partially use amino acids for energy, these being reserves only used in case of starving or at reproduction. This would point to the use of other reserves before protein and amino acids at early stages of life. Fast-growing cephalopods, when fed properly, are extremely efficient in converting food to protein, display low protein degradation and exhibit increased efficiency of retaining synthesized protein (Carter et al. 2009). On the other hand, cephalopods are said to have a lim-ited capacity for lipid oxidation (Ballantyne et al. 1981), and its digestion becomes slow and inefficient due to the lack of emulsifiers (biliary salts) in the digestive tract (Vonk 1962). On the contrary, Moltschaniwskyj and Johnston (2006) have shown that *Euprymna tasmanica* has the ability to digest lipids (very high levels of lipase were found in the digestive gland) but these are not stored in the digestive gland, which indicates the species capacity of using lipids as a source of fuel (Swift et al. 2005). This species is known to have a very sedentary lifestyle (it does not move a lot, so there are not many mantle burst activities) in Southern Australia temperate waters. A similar ability to use lipids had already been displayed by individuals of *S. officinalis* from the English Channel populations, which metabolized lipids instead of protein or CHs when facing prolonged starvation (Castro et al. 1992). On the other hand, Lamarre et al. (2012) observed the mixed use of lipids and protein, and after 8 days the exclusive use of protein in short starvation, in cuttlefish from the Mediterranean Sea. This use of lipids by cuttlefish might be eventually identical, at the cellular level, to what is described by Finn and Dice (2006) in vertebrates.

The higher growth rates observed in cephalopods that live or are cultured in sub-tropical regions (with high temperatures than those observed in temperate waters) would mean that from a given temperature threshold, cephalopods would use CHs as energy, while at lower temperatures, they would preferably use other sources such as lipids and protein (depending on food availability). The enzyme content of the

different life stages helps to understand the eventual existence of different metabolisms. While the enzyme content of the English Channel population of *S. officinalis* has been reported, for several years and by numerous researchers, those of Faro or Mediterranean populations have never, to the best of our knowledge, been reported. Regarding the first, Boucaud-Camou (1969, 1947) characterized amylase and protease activities in different digestive organs of juvenile cuttlefish and Yim (1978) detected amylase activity in mature cuttlefish. According to Koueta et al. (2000), this activity increases with age, not being present at hatching, which is concomitant with the maturation of the sepia digestive system (Boucaud-Camou et al. 1985). Nonetheless, the use of silage as enrichment for shrimp given as prey to cuttlefish hatchlings promoted an increase of total CH and peptides in the diet and supported 100 % survival plus increased growth, when compared with natural diets, and despite the lower content in total protein (Le Bihan et al. 2006). However, these same authors reported an inhibition of amylase activity but higher proteolytic activity.

Higher temperatures promote oxygen-efficient adenosine triphosphate (ATP) production due to limitations in available oxygen (Hochachka 1994; Pörtner 2010). However, most studies on cephalopod metabolism have been performed in fasting animals, where the stressful situation of meeting the energy requirements will promote the use of protein reserves (McCue 2010). In addition, one has to consider that all animals exhibit adaptive biochemical and physiological responses to the lack of food (Wang et al. 2006). This is particularly true regarding cephalopods, most of which inhabit environments in which food availability fluctuates or encounters with appropriate food items might be rare and unpredictable at given geographical locations or seasons.

Houlihan et al. (1990) studied protein metabolism in *O. vulgaris* and concluded that the high growth rates displayed by this species rely on high rates of protein synthesis and high efficiencies of retention of synthesized protein and little protein degradation. According to Oellermann et al. (2012), the European cuttlefish has the capability of adjusting its cellular and mitochondrial energetics over short- and long-term changes of temperature and environmental conditions, which is an evolutionary adaptation of given populations, such as *S. officinalis*. In addition, temperature has a significant effect on oxygen consumption (Grigoriou and Richardson 2009) and energy metabolism of cuttlefish (Mark et al. 2008). Furthermore, temperate cuttlefish (English Channel populations) display a predominant oxidation of proline in systemic heart, while subtropical cuttlefish (Mediterranean populations) exhibit enhanced pyruvate oxidation. The latter is supported by the findings of Ballantyne et al. (1981) on octopine dehydrogenase linking amino acid (arginine) and CH (pyruvate) metabolism, which are said to occur during hypoxic conditions, burst activity or both.

In this sense, cold-adapted metabolism in cuttlefish will show a suppressed CH metabolism, favoring the use of lipids (e.g. results of Koueta et al. (2002); Perrin et al. (2004) and Koueta et al. (2006)) and proline, which are less oxygen efficient (Hochachka 1994). At lower temperatures, amino acids such as glutamate, ornithine or arginine may sustain the supply of succinate (Ballantyne et al. 1981; Hochachka et al. 1983). Cephalopods should have developed an aerobic CH–amino acid metabo-

lism that maximizes ATP yield per unit of consumed oxygen due to the lack of an intracellular myoglobin analogue and the exclusive localization of mitochondria in interfibrillar zones (Hochachka 1994; Ballantyne 2004). CH are catabolized rapidly following ingestion, and are not used during fasting, but mobilized for locomotor activity (O'Dor et al. 1984). According to Hochachka and Fields (1982) and Storey and Storey (1978), glucose metabolism may be coupled to that of proline through glucose conversion to pyruvate, which is oxidized in the Krebs cycle, and proline simultaneously augments the cycle intermediates. These first authors still add that glutamate, proline and most probably arginine can be used directly as gluconeogenic precursors.

5.9   Conclusions

Proteins are the most abundant macronutrient in cephalopods, and large protein and amino acid contents in the diet are required for sustaining growth and eventually fulfilling energy demands. Although low in quantitative terms, lipids are essential in cephalopod nutrition, with long-chain PUFA playing a pivotal role, since the enzymatic machinery unveiled through molecular research points at their inability in the synthesis of these important nutrients. Therefore, the effect of dietary protein/lipid ratios on amino acid absorption efficiency and metabolism needs to be researched.

Cephalopods also require an adequate provision of antioxidants like pro-vitamin A and carotenoids, since the importance of vitamins in photoreception, growth and development must not be neglected. It is generally accepted that cephalopods do not have a specific requirements for dietary CH, but it has been reported that they are able to rapidly catabolize dietary CH to account for energy demands in explosive activities such as prey capture and fleeing from predators, and the existence of a CH metabolism has been established. However, the CH theory presented in this chapter needs to be proven. Finally, it is accepted that octopuses and cuttlefish, as carnivorous species, meet the majority of their elemental requirements from the diet, although direct uptake from the seawater has also been shown to occur through an ion balance mechanism regulated by the digestive gland appendages.

Methods for the determination of possible metabolic pathways have been developed for fish larvae (Conceição et al. 2003; Morais et al. 2004) and these may be adapted for the study of metabolism in cephalopods. Additionally, the use of systems biology modelling to cephalopod nutrition, similar to that published by Hormiga et al. (2010), might help on a faster progress towards our understanding of cephalopods' physiology and nutrition.

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