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Growth and survival of cuttlefish (*Sepia officinalis*) of different ages fed crustaceans and fish. Effects of frozen and live prey

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Abstract

Three feeding experiments, using live mysid shrimp, grass shrimp or fish fry as prey for 1-, 30- and 60-day-old cuttlefish were conducted to determine the efficiency of each dietary source in relation to cuttlefish size and age. Additionally, a fourth experiment using fish fry and grass shrimp, but previously frozen, was also conducted. The results showed that when 1-day-old cuttlefish were fed mysids, grass shrimp or fish for 4 weeks, mysids were the best prey, but only during the first week. From this moment until the end of the experiment, the best growth rate was when cuttlefish were fed grass shrimp. Cuttlefish fed fish fry showed the poorest growth rate throughout the experiment. Similarly, cuttlefish aged 30 or 60 days fed grass shrimp or fish fry had the best growth rates when fed grass shrimp. When cuttlefish were fed live fish, survival increased with size of cuttlefish (73.3%, 91.7% and 100% for 1, 30 and 60 days cuttlefish, respectively). In the fourth experiment, using frozen diets, overall acceptance of each diet (feeding rates) was the same for fish and shrimp. However, lower growth was obtained when cuttlefish were fed fish compared to grass shrimp. This lower growth was due to a lower food conversion (28% vs. 41%). Since cephalopod paralarvae and juvenile most likely need prey rich in polyunsaturated fatty acids (PUFA), phospholipids and cholesterol, and a moderate content in neutral lipids, we have analyzed the

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biochemical compositions of the different prey to evaluate the influence of this factor on growth and survival.

Keywords: Age; Biochemical composition; Cuttlefish; Feeds; n-3 HUFA

1. Introduction

Cephalopods are characterized by short life cycles, fast growth rates and high food conversion (Boletzky, 1983; Lee, 1994; Domingues et al., 2001a). Some species present feeding rates that vary between 20% and 50% body weight per day (bw day^{-1}) (Boucher-Rodoni et al., 1987; Domingues et al., 2001a). Typical growth rates vary between 3% and 10% bw day^{-1} throughout the life cycle (Forsythe and Van Heukelem, 1987). During the past few years, there has also been an increase cephalopod market prize (Navarro and Villanueva, 2000). These characteristics make some cephalopod species promising animals for commercial culture (Forsythe et al., 1991; Lee et al., 1998).

Cuttlefish (*Sepia officinalis*) is relatively easy to culture since it easily reproduces in captivity, has large eggs and hatchlings (Zahn, 1979). Hatchling survival is high, as long as appropriate live food is supplied (Pascual, 1978; Forsythe et al., 1994; Domingues et al., 2001b, 2002). Cuttlefish are also resistant to disease, crowding and handling (Forsythe et al., 1994). These characteristics give this species a high potential for large-scale culture. Because of this, the cuttlefish has been cultured for the last decades (Choe, 1966; Richard, 1971, 1975; Pascual, 1978; DeRusha et al., 1989; Forsythe et al., 1994; Lee et al., 1998; Domingues et al., 2001b, 2002), and is one of the most well-known and easily cultured cephalopods (Forsythe et al., 1994; Domingues, 1999; Domingues et al., 2001b).

Cuttlefish are known to change their diet in the wild, with the importance of fish increasing and crustacean decreasing, during growth (Castro and Guerra, 1990). However, several authors have reported a lower growth and survival when using live or frozen fish to feed cuttlefish compared to when crustaceans are used (Pascual, 1978; DeRusha et al., 1989; Domingues et al., in press). During the present work, live fish fry or crustaceans were fed to cuttlefish aged 1, 30 and 60 days, in order to determine the influence of these prey on growth and survival as cuttlefish grow larger.

Since some authors could raise the question that growth when feeding live fish was lower due to the higher difficulty to capture fish compared to shrimp, another experiment was conducted, using both frozen shrimp and fish. This eliminated the degree of difficulty associated to prey capture.

Research on lipid and fatty acid requirements for cephalopods have been neglected because they have a mainly protein metabolism. Nevertheless, cephalopod paralarvae and juvenile most likely need prey rich in polyunsaturated fatty acids (PUFA), phospholipids and cholesterol, and a moderate content in neutral lipids (Navarro and Villanueva, 2000; Koueta et al., 2002; Domingues et al., in press). Therefore, in the present study, the biochemical composition of the different prey used to fed cuttlefish were also analyzed in order to get a better evaluation of dietary sources in relation with cuttlefish age.

2. Material and methods

2.1. Experiment I

The first experiment was conducted to determine the effects of feeding different live prey on growth and survival of cuttlefish hatchlings (newly born) during 4 weeks. The three prey used in the experiments were (1) mysid shrimp (*Paramysis nouvelli*), (2) grass shrimp (*Palaemonetes varians*) and (3) fish fry (*Atherina* sp.). All live prey used here were captured in the ponds surrounding the research facility. During the experiment, all cuttlefish were fed ad libitum, twice a day, at 1000 and 1600 h. Average weight of mysids, grass shrimp and fish fry fed to the cuttlefish was 7 ± 0.6 mg ($n = 230$), 28 ± 9 mg ($n = 250$), 12 ± 5 g ($n = 230$), respectively. Cuttlefish used in the experiment were part of a fourth generation (F4) cultured in our research facility and all of them hatched the same day. Three replicates of 30 hatchlings each were used for each of the three diets tested. Average weight of the 270 hatchlings (30 hatchling in each of the 9 replicates) used was 99 ± 14 mg. No significant differences ($P > 0.05$) in weight were found among the nine replicates at the start of the experiment. Cuttlefish were weighed individually every 7 days and data were used to calculate: (1) average weight and (2) instantaneous growth rate (GR) (% body weight day^{-1})=$((\ln W_2 - \ln W_1)/t \times 100$, where W_2 and W_1 are the final and initial weight of the cuttlefish, respectively, ln the natural logarithm and t the number of days of the experimental period.

During the experiment, a flow-through system was used. This was composed of 12 plastic rectangular tanks (38 × 28.5 cm), with a water depth of 12 cm. Total volume in each tank averaged 10 l. Water flow was 10 l h^{-1}. During the experiment, 3 of the 12 tanks were not used. Water temperature was measured every day, while salinity and dissolved oxygen were measured on a weekly basis.

2.2. Experiment II

The second experiment tested the effects of feeding two different prey (grass shrimp or fish fry) to cuttlefish aged 30 days during 4 weeks. Average weight of grass shrimp and fish fry fed to the cuttlefish was 122 ± 54 mg ($n = 200$) and 141 ± 84 mg ($n = 200$), respectively. Three replicates of 20 cuttlefish each were used for each of the two diets tested. Average weight of the 120 cuttlefish (six replicates, three per diet) was 600 ± 105 mg. No significant differences ($P > 0.05$) in weight were found among the six replicates at the start of the experiment. Culture and feeding conditions were carried on as in experiment I. Therefore, 6 of the 12 tanks of the system were not used. All cuttlefish used in this experiment were previously fed during their first 30 days until the beginning of the experiment with mysid shrimp (first week) and grass shrimp (the rest of the time).

2.3. Experiment III

The third experiment tested the effects of feeding the same two diets as in experiment II (grass shrimp or fish fry) to cuttlefish aged 60 days during 4 weeks. Average weight of grass shrimp and fish fry fed to the cuttlefish was 154 ± 98 mg ($n = 200$) and 225 ± 104 mg

($n = 200$), respectively. Six replicates of six cuttlefish were used for each of the two diets tested. Average weight of the 72 cuttlefish (12 replicates) was 5.73 ± 0.07 g. No significant differences ($P>0.05$) in weight were found among the 12 replicates at the start of the experiment. Culture and feeding conditions were the same as for experiments I and II. In this case, all 12 tanks from the system were used. All cuttlefish used in this experiment were fed during their first 60 days until the start of the experiment with mysid shrimp (first week) and grass shrimp (the rest of the time).

2.4. Experiment IV

A fourth experiment tested the effects of two frozen diets (fish and shrimp), but in this case, prey were previously frozen and fed to cuttlefish within 3 days after freezing. The experiment lasted for 5 weeks (35 days). A total of six replicates (three replicates for each of the two diets tested) were used, with 15 cuttlefish (50 days old) in each. Average weight of the 90 cuttlefish from all replicates was 2.15 ± 0.36 g. No significant differences ($P>0.05$) in weight were found among the six replicates at the start of the experiment. Culture conditions and weighing procedures were similar as in the previous experiments. All cuttlefish used in this experiment were fed during their first 50 days with mysid shrimp (first week) and grass shrimp (the rest of the time).

Cuttlefish in each replicate were fed 20% bw day^{-1}. Food was weighed and presented equally, three times per day (0900, 1200 and 1600 h). After 1 h, food remains were removed and weighed, and food eaten calculated. Food eaten was registered for each feeding period. Mortality was determined for each weighing interval. Cuttlefish were weighed individually every 7 days and data were used to calculate growth and instantaneous growth rate. Since during this experiment, food eaten was determined, we also calculated: (1) feeding rate (FR) (% body weight day^{-1})=(FI/average $W(t)$) × 100, where FI is the food ingested and average $W(t)$ is the average wet weight of the cuttlefish during the time period (t); and (2) food conversion (FC)=$(W_2 - W_1)$/FI, where $W_2 - W_1$ is the weight gained by the cuttlefish during the time period.

Temperature in the first three experiments varied between 20 ± 2 °C, always increasing from the start to the end of the experiments. During experiment IV, average temperature gradually increased from 24 to 25 °C. Salinity was 36 ± 1 ‰, and dissolved oxygen varied between 70% and 80% in all experiments. During all experiments, and for every weighing period, every ANOVA comparison between the replicates fed each diet showed that there were no significant differences ($P>0.05$) between them. Therefore, cuttlefish from the replicates fed the same diet in each experiment were pooled.

2.5. Statistical analysis

After weighing periods, statistical analysis was performed to determine differences in weight among groups fed the different diets. ANOVAs followed by Tukey multiple comparisons tests (Sokal and Rohlf, 1981) were performed among the different replicates of cuttlefish fed each diet in each experiment. If no significant differences were found among replicates fed the same diet, all cuttlefish fed that same diet were pooled, and a Student's t-test (Zar, 1984) was performed to compare differences in weight between the

diets. Also, nonparametric Man–Whitney *U*-tests (Zar, 1984) were conducted to determine differences in growth rates for all experiments, as well as for feeding rates and food conversions in experiment IV.

2.6. Dietary analysis

Moisture content from different prey was determined from 500-mg samples using the method of Horwitz (1980). Protein content was determined according to the Kjeldahl method (AOAC, 1985). Total carotenoids were extracted using the method of Barua et al. (1993). Total lipid was extracted with chloroform/methanol (2:1, v/v) containing 0.01% of butylated hydroxytoluene (BHT) as antioxidant (Christie, 1982). The organic solvent was evaporated under a stream of nitrogen and the lipid content determined gravimetrically. Lipid classes were separated by one-dimensional double-development high-performance thin layer chromatography (HPTLC) using methyl acetate/isopropanol/chloroform/methanol/0.25% (w/v) KCl (25:25:25:10:9 by volume), as the polar solvent system and hexane/diethyl ether/glacial acetic acid (80:20:2 by volume), as the neutral solvent system. Lipid classes were quantified by charring with a copper acetate reagent followed by calibrated scanning densitometry using a Shimadzu CS-9001PC dual wavelength flying spot scanner (Olsen and Henderson, 1989). Total lipid (TL) extracts were subjected to acid-catalyzed transmethylation for 16 h at 50 °C, using 1 ml of toluene and 2 ml of 1% sulphuric acid (v/v) in methanol. The resultant fatty acid methyl esters (FAME) were purified by thin layer chromatography (TLC), and visualized under UV light with 2′,7′-dichlorofluorescein in 98% (v/v) methanol, containing 0.01% BHT (Christie, 1982). FAME were separated and quantified by using a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (250 °C) and a fused silica capillary column Supelcowax™ 10 (30 m × 0.32 mm I.D.). Helium was used as carrier gas and the oven initial temperature was 180 °C for 10 min, followed by an increase at a rate of 2.5 °C min^{-1} to a final temperature of 215 °C. Individual FAME were identified by reference to authentic standards and to a well-characterized fish oil.

Carotenoids were extracted from the same samples, according to the method of Barua et al. (1993). Samples (1 g) were homogenised with ethyl acetate/ethanol (10 ml, 1:1, v/v) and centrifuged (150 × *g*, 2 min), the supernatant was decanted into a clean tube. The pellet was re-centrifuged in ethyl acetate (5 ml) and re-centrifuged and the supernatant was combined with the first supernatant. Finally, the pellet was re-homogenized in 10 ml of hexane and re-centrifuged and the supernatant was combined with the pooled supernatant. The pooled supernatant was dried under a stream of nitrogen and vacuum-desiccated for 1 h. After desiccation, 2 ml of hexane containing 0.01% BHT was added to the residue. The final solution was centrifuged (5000 × *g*, 5 min) and the total carotenoids content from the supernatant were measured by spectrophotometer (Shimadzu, UV-120-02) at 470 nm against a hexane (+BHT) blank, using the $E_{1\%,1\ cm}$ of 2100. Total contents of carotenoids are expressed as μg of carotenoids/g of tissue (dry weight basis).

BHT, potassium chloride, potassium bicarbonate and 2′,7′-dichlorofluorescein were supplied by Sigma (St. Louis, MO). TLC (20 × 20 cm × 0.25 mm) and HPTLC (10 × 10 cm × 0.15 mm) plates, precoated with silica gel (without fluorescent indicator), were purchased from Machery-Nagel (Düren, Germany). All organic solvents used were of

reagent grade and were purchased from Panreac (Barcelona, Spain). Results are presented as means ± S.D. from the analyses of triplicate experiments.

3. Results

3.1. Experiment I

During this experiment, survival of newly born hatchlings fed mysids was 91.1%, with mortality occurring only during the last week. Hatchlings fed fish had 73.3% survival at the end of the experiment, but hatchling mortality occurred only between weeks 2 and 3. None of the 90 hatchlings fed grass shrimp died during the 4 weeks of the experiment.

Growth and growth rates of hatchlings fed the 3 diets are shown in Table 1. At the end of the experiment, weight of hatchlings fed each diet was significantly different ($P<0.05$), being the ones fed grass shrimp the largest, followed by the ones fed mysids. Lowest growth was obtained when feeding fish. Growth from hatchlings fed fish was lower ($P<0.05$) even at the end of the first week, while hatchlings fed grass shrimp were larger ($P<0.05$) than the ones fed mysids, from the end of the third week onwards.

Average growth rates for the experiment were 6.2 ± 0.7%, 7.5 ± 1.4% and 2.9 ± 0.7% bw day^{-1} for hatchlings fed mysids, grass shrimp and fish, respectively, and they were significantly different ($P<0.05$) between the 3 diets. Growth rates were always different between diets and in every weighing period.

3.2. Experiment II

None of the cuttlefish fed shrimp died during the experiment. Survival of cuttlefish fed fish was 91.7%, with mortality (five cuttlefish between the three replicates) occurring only during the last week.

Table 1
Growth and growth rates of newly born cuttlefish fed live mysids, shrimp or fish during 4 weeks

Prey	Days				
	Day 1	Day 8	Day 15	Day 22	Day 29
Weight (mg)					
Mysids	100 ± 14	154 ± 42	250 ± 56	360 ± 76*	496 ± 86*
Grass shrimp	96 ± 12	146 ± 34	257 ± 55	491 ± 88*	796 ± 144*
Fish	98 ± 15	120 ± 30*	136 ± 43*	173 ± 51*	209 ± 54*
GR (% bw day^{-1})					
Mysids		6.4 ± 0.4*	7.1 ± 0.4*	6.1 ± 0.2*	5.3 ± 0.2*
Grass shrimp		5.5 ± 0.4*	8.4 ± 0.1*	8.6 ± 0.3*	7.5 ± 0.1*
Fish		3.0 ± 0.4*	1.8 ± 0.1*	3.2 ± 0.1*	3.4 ± 0.0*

* Values were significantly different within that period.

Table 2
Growth and growth rates of cuttlefish aged 30 days, fed either live shrimp or fish during 4 weeks

Prey	Days				
	Day 1	Day 8	Day 15	Day 22	Day 29
Weight (g)					
Grass shrimp	0.61 ± 0.09	0.95 ± 0.15*	1.39 ± 0.23*	2.03 ± 0.28*	2.96 ± 0.59*
Fish	0.60 ± 0.12	0.70 ± 0.14*	0.91 ± 0.17*	0.99 ± 0.26*	1.15 ± 0.15*
GR (% bw day^{-1})					
Grass shrimp		6.1 ± 0.0*	5.8 ± 0.0*	5.5 ± 0.1*	5.3 ± 0.1*
Fish		2.2 ± 0.0*	2.8 ± 0.0*	2.4 ± 0.1*	2.2 ± 0.0*

* Values were significantly different within that period.

Growth and growth rates of cuttlefish fed the two diets are shown in Table 2. For every weighing period, even at the end of the first week, cuttlefish fed grass shrimp were larger ($P < 0.05$) than the ones fed fish.

Average growth rates for the experiment were 5.7 ± 0.4% and 2.4 ± 0.3% bw day^{-1} for cuttlefish fed grass shrimp and fish, respectively, and were significantly different ($P < 0.05$) at every weighing period (Table 2).

3.3. Experiment III

No cuttlefish from any replicate testing both diets died during the experiment.

Growth and growth rates of cuttlefish fed the two diets are shown in Table 3. Cuttlefish fed grass shrimp were larger ($P < 0.05$) than the ones fed fish in every weighing interval, even at the end of the first week.

Average growth rates for the experiment were 5.8 ± 0.1% and 3.5 ± 1.3% bw day^{-1} for cuttlefish fed grass shrimp and fish, respectively, and were significantly different ($P < 0.05$) at every weighing period (Table 3).

When comparing growth of cuttlefish aged 1, 30 or 60 days and fed live grass shrimp, growth rates of newly born hatchlings (7.5 ± 1.4% bw day^{-1}) were higher ($P < 0.05$) compared to 30- or 60-day-old cuttlefish (5.7 ± 0.4% and 5.8 ± 0.1% bw day^{-1}, respectively), but they were not different ($P > 0.05$) between cuttlefish aged 30 and 60 days.

Table 3
Growth and growth rates of cuttlefish aged 60 days, fed either live shrimp or fish during 4 weeks

Prey	Days				
	Day 1	Day 8	Day 15	Day 22	Day 29
Weight (g)					
Grass shrimp	5.75 ± 0.06	8.59 ± 0.44*	12.83 ± 1.09*	19.48 ± 2.91*	29.05 ± 3.74*
Fish	5.71 ± 0.08	6.43 ± 0.35*	8.07 ± 1.47*	10.86 ± 3.02*	14.89 ± 4.96*
GR (% bw day^{-1})					
Grass shrimp		5.7 ± 0.2*	5.9 ± 0.4*	6.0 ± 0.1*	5.8 ± 0.2*
Fish		1.6 ± 0.2*	3.5 ± 0.2*	4.2 ± 0.4*	4.7 ± 0.2*

* Values were significantly different within that period.

3.4. Experiment IV

Survival of cuttlefish fed grass shrimp was 80%. During the third week, three cuttlefish died (one in each replicate), and six more died during the last week (two in each replicate). As for survival of cuttlefish fed fish, it was 91.9%. Only four cuttlefish fed this diet died during the experiment, with one dying between the second and third weeks, and the remaining three during the last week (one in each replicate).

Growth and growth rates, feeding rates and food conversions of cuttlefish fed the two diets are shown in Table 4. Even from the end of the first week, cuttlefish fed frozen grass shrimp were always larger ($P<0.05$) than the ones fed frozen fish.

Average growth rates for the experiment were $5.1 \pm 1.6\%$ and $3.3 \pm 1.5\%$ bw day^{-1} for cuttlefish fed frozen grass shrimp or fish, respectively, and were significantly different ($P<0.05$). Growth rates were always significantly different ($P<0.05$) for every weighing interval (Table 4).

Average feeding rates for this experiment were $12.4 \pm 1.8\%$ and $11.7 \pm 2.7\%$ bw day^{-1} for cuttlefish fed frozen grass shrimp or fish, respectively, and they were not significantly different ($P>0.05$). Cuttlefish fed frozen grass shrimp had higher feeding rates ($P<0.05$) during the first 2 weeks, but for the last 3 weeks, there were no differences ($P>0.05$) between feeding rates (Table 4).

Average food conversions for the experiment were 0.41 ± 0.05 and 0.28 ± 0.09 for cuttlefish fed frozen grass shrimp or fish, respectively, and were significantly different ($P<0.05$). From the start of the experiment, cuttlefish fed frozen grass shrimp had always higher ($P<0.05$) food conversions than the ones fed frozen fish.

Table 4
Growth and growth rates, feeding rates and food conversions of cuttlefish aged 50 days, fed either frozen shrimp or fish during 5 weeks

Prey	Days					
	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36
Weight (g)						
Grass shrimp	2.2 ± 0.5	2.7 ± 0.8*	4.7 ± 1.2*	6.5 ± 1.6*	9.2 ± 2.4*	13.1 ± 4.0*
Fish	2.2 ± 0.2	2.3 ± 0.7*	3.3 ± 1.0*	4.0 ± 1.2*	5.4 ± 1.6*	6.8 ± 2.1*
GR (% bw day^{-1})						
Grass shrimp		3.3 ± 0.5*	7.7 ± 0.7*	4.6 ± 0.7*	5.0 ± 0.2	5.1 ± 0.4*
Fish		1.1 ± 0.7*	5.0 ± 0.3*	2.6 ± 0.7*	4.2 ± 0.2	3.5 ± 0.1*
FR (% bw day^{-1})						
Grass shrimp		10.4 ± 1.1*	15.3 ± 0.7*	12.7 ± 0.4	12.1 ± 0.7	11.7 ± 1.8
Fish		7.4 ± 0.8*	13.2 ± 0.4*	13.9 ± 0.9	13.1 ± 0.4	10.8 ± 0.6
Food conversions						
Grass shrimp		0.37 ± 0.02*	0.50 ± 0.06*	0.36 ± 0.05*	0.41 ± 0.02*	0.43 ± 0.05*
Fish		0.17 ± 0.09*	0.38 ± 0.03*	0.18 ± 0.04*	0.32 ± 0.01*	0.32 ± 0.02*

* Values were significantly different within that period.

3.5. Dietary analysis

Proximate composition of three prey used in these experiments is shown in Table 5. Total protein content was lower in mysid shrimp (66.90 ± 7.78), whereas grass shrimp and fish fry had similar values (77.08 ± 2.39 and 76.16 ± 18.88, respectively). However, the high variability observed, particularly in fish fry and mysid shrimp, prevents stronger conclusions on the total protein composition of the three prey. On the other hand, grass shrimp showed the highest level of total carotenoid content (0.24 ± 0.04 μg/g, DWB) and fish fry the lowest level (0.02 ± 0.02 μg/g, DWB). Mysid shrimp displayed a mean value of 0.13 ± 0.00 μg/g, DWB. When analyzing the lipid composition, similar values in the total (TL), neutral (NL) and polar lipid (PL) content among the three diets can be observed, with a high variability in the fish fry. The lipid classes composition also shows the high variability described for fish fry TL content, especially in triacylglycerol (TG), esterol ester (EE) and phosphatidylcholine (PC). Among the three diets, the lower content of cholesterol (CHO) in mysid shrimp and the high levels of EE in fish fry with respect to both shrimp species analyzed are remarkable. The high content of free fatty acids (FFA) found in mysid shrimp compared with the other two diets is also noticeable.

Fatty acid composition of total lipid from the three diets used in the experiments is shown in Table 6. Results indicate similar levels for some of the major fatty acid groups, such as saturates, monoenes, n-3 and n-6 series for all diets. However, when analyzing the

Table 5
Composition of proteins (% dry weight basis (DWB)), total carotenoid contents (μg/g, DWB), moisture (%), total lipids (% DWB) and lipid classes (% of total lipid) of live diets for cuttlefish hatchlings

Live diets	Mysid shrimp, *P. nouvelli*	Grass shrimp, *P. varians*	Fish fry, *Atherina* sp.
Crude protein	66.90 ± 7.78	77.08 ± 2.39	76.16 ± 18.88
Total carotenoid	0.13 ± 0.00	0.24 ± 0.04	0.02 ± 0.02
Moisture	79.54 ± 0.48	79.26 ± 0.32	76.38 ± 1.64
Total lipid	9.11 ± 0.90	8.42 ± 0.61	10.87 ± 6.21
Sphingomyelin	0.36 ± 0.10	0.0	1.83 ± 0.59
Phosphatidylcholine	13.36 ± 0.67	14.50 ± 1.03	13.41 ± 3.68
Phosphatidylserine	5.99 ± 0.32	4.75 ± 0.10	4.44 ± 1.37
Phosphatidylinositol	1.03 ± 0.04	1.86 ± 0.22	1.44 ± 0.06
Phosphatidylglycerol[a]	4.52 ± 1.13	1.88 ± 0.41	2.68 ± 0.79
Phosphatidylethanolamine	13.07 ± 1.70	19.25 ± 0.62	10.82 ± 1.88
Diacylglycerol	2.58 ± 0.39	0.0	0.73 ± 0.42
Cholesterol	18.42 ± 1.20	33.49 ± 0.69	24.37 ± 2.67
Free fatty acids	11.12 ± 1.13	0.82 ± 0.12	4.31 ± 1.58
Triacylglycerol	26.82 ± 4.63	21.45 ± 1.01	28.64 ± 13.32
Esterol ester	2.72 ± 0.59	2.00 ± 0.07	7.34 ± 2.14
Neutral lipids	61.67 ± 3.68	57.76 ± 0.51	65.40 ± 8.03
Polar lipids	38.33 ± 3.68	42.24 ± 0.51	34.60 ± 8.03

Results represent means ± S.D. ($n = 3$).
[a] Contains phosphatidylglycerol, phosphatidic acid and cardiolipin.

Table 6
Fatty acid composition of total lipid (% of lipid weight) from different live prey used as food for cuttlefish hatchlings

Live diets	Mysid shrimp, *P. nouvelli*	Grass shrimp, *P. varians*	Fish fry
14:0	2.03 ± 0.31	2.49 ± 0.21	1.92 ± 0.06
15:0	3.31 ± 0.26	0.64 ± 0.03	0.70 ± 0.07
16:0	20.23 ± 2.71	18.20 ± 0.44	20.79 ± 0.48
16:1[a]	5.02 ± 1.40	6.39 ± 0.55	3.98 ± 0.28
18:0	3.63 ± 0.39	8.99 ± 0.06	12.03 ± 0.10
18:1n-9	5.39 ± 0.51	4.31 ± 0.33	7.20 ± 0.49
18:1n-7	3.24 ± 0.34	8.02 ± 0.12	3.74 ± 0.14
18:2n-6	1.78 ± 0.05	2.00 ± 0.27	1.15 ± 0.27
18:3n-3	2.19 ± 0.20	1.32 ± 0.14	0.73 ± 0.21
18:4n-3	1.02 ± 0.27	0.86 ± 0.15	1.01 ± 0.18
20:1[b]	1.54 ± 0.32	0.09 ± 0.16	0.22 ± 0.31
20:4n-6	3.88 ± 0.74	4.79 ± 0.92	4.33 ± 0.27
20:4n-3	0.14 ± 0.12	0.25 ± 0.06	0.15 ± 0.21
20:5n-3	21.38 ± 2.82	20.71 ± 0.84	9.10 ± 0.69
22:5n-6	0.81 ± 0.08	1.29 ± 0.05	0.72 ± 0.08
22:5n-3	0.98 ± 0.20	0.64 ± 0.04	2.38 ± 0.24
22:6n-3	16.32 ± 2.05	13.73 ± 0.31	24.31 ± 0.37
Unknown	1.15 ± 0.47	1.29 ± 0.31	1.54 ± 0.82
Totals			
Saturates[c]	30.49 ± 3.51	32.34 ± 0.62	36.73 ± 0.03
Monoenes[d]	15.44 ± 2.18	19.22 ± 0.07	15.95 ± 0.20
n-3[e]	44.80 ± 5.01	38.05 ± 0.98	38.35 ± 1.34
n-6[f]	7.55 ± 0.77	8.81 ± 1.12	7.06 ± 0.25
n-3 HUFA	39.29 ± 5.00	35.40 ± 0.99	36.20 ± 1.00
n-3/n-6	5.98 ± 0.96	4.38 ± 0.68	5.44 ± 0.39
DHA/EPA[g]	0.76 ± 0.05	0.66 ± 0.02	2.68 ± 0.16
EPA/AA[h]	5.64 ± 1.21	4.45 ± 0.97	2.11 ± 0.29

Results represent means ± S.D. ($n = 3$).
[a] Contains n-9 and n-7 isomers.
[b] Contains n-11 and n-9 isomers. Totals include some minor components not shown.
[c] Includes 17:0 and 20:0.
[d] Includes 14:1, 17:1n-7 and 22:1.
[e] Includes 20:3n-3 and 21:5n-3.
[f] Includes 18:3n-6, 20:2n-6 and 22:4n-6.
[g] 22:6n-3/20:5n-3.
[h] 20:5n-3/20:4n-6; 0.0, values $\leq 0.3\%$.

percentage of the n-3 HUFA, it is relevant to indicate that the DHA/EPA ratio is clearly higher in fish fry compared to the crustacean species. The values obtained are due to the higher levels of eicosapentaenoic acid (EPA, 20:5n-3) and lower levels of docosahexaenoic acid (DHA, 22:6n-3) found in the crustacean species analyzed. Another important result obtained is related to the EPA/arachidonic acid (AA, 20:4 n-6) ratio, which is higher the mysid and grass shrimp, compared to fish fry.

Comparison between lipid classes of live and frozen grass shrimp (placed for 3 days in a −20 °C freezer) is shown in Table 7. The freezing procedure lowered ($P < 0.05$) the

Table 7
Total carotenoid contents (μg/g, DWB), moisture (%), total lipids (% DWB) and lipid classes (% of total lipid) of frozen shrimp and live shrimp

Diets	Frozen shrimp	Live shrimp
Total carotenoid	0.20 ± 0.02	0.24 ± 0.04
Moisture	80.62 ± 1.75	79.26 ± 0.32
Total lipid	10.05 ± 2.32	8.42 ± 0.61
Sphingomyelin	0.0	0.0
Phosphatidylcholine	11.31 ± 2.15	14.50 ± 1.03
Phosphatidylserine	3.70 ± 0.59	4.75 ± 0.10
Phosphatidylinositol	1.45 ± 0.24	1.86 ± 0.22
Phosphatidylglycerol[a]	3.42 ± 0.39	1.88 ± 0.41*
Phosphatidylethanolamine	12.92 ± 0.89	19.25 ± 0.62*
Cholesterol	32.83 ± 2.44	33.49 ± 0.69
Free fatty acids	3.62 ± 1.09	0.82 ± 0.12*
Triacylglycerol	29.39 ± 6.62	21.45 ± 1.01
Esterol ester	1.34 ± 0.48	2.00 ± 0.07
Neutral lipids	67.19 ± 3.17	57.76 ± 0.51*
Polar lipids	32.81 ± 3.17	42.24 ± 0.51*

Results represent means ± S.D. ($n = 3$).
Pairs of means were compared by Student's *t*-test.
[a] Contain phosphatidylglycerol, phosphatidic acid and cardiolipin.
* Significantly different ($P < 0.05$).

concentration of polar lipids and increased the percentage of neutral lipid. Among the lipid classes, only the free fatty acid (FFA) content was higher in the frozen shrimp, while phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) were higher in the live shrimp.

4. Discussion

Several authors have reported feeding mysids during the early stages of the life cycle of the cuttlefish. Pascual (1978) used mysids as the first food for cuttlefish during a month, as we did in this experiment. The most important finding obtained from experiment I was that mysids are indeed the best first-food for cuttlefish hatchlings. Nevertheless, the analysis of Table 1 shows that mysids promoted better growth only during the first week of their lives, where average growth rates were significantly higher ($P < 0.05$) compared to when feeding grass shrimp. However, during the second week, hatchlings feeding on grass shrimp increased considerably their growth rates, to an extent that growth rates during this second week and until the end of the experiment were significantly higher ($P < 0.05$) than those obtained when feeding mysids. Until the present experiment, our culture protocol for cuttlefish was to feed them mysids until 2 weeks old (Forsythe et al., 1994).

The decrease in growth rates of cuttlefish fed mysids compared to grass shrimp after the second week of their lives is, in our opinion, due to the size of the prey and not the quality of

the food. In fact, at 1 week old, hatchlings can easily capture grass shrimp larger than them, and several times larger than mysids. At this age and size, cuttlefish have to capture considerably more mysids to cope with their energy requirements, and this implies also more energy spent hunting, while those feeding on larger prey (grass shrimp) hunt less, and obtain more food. Contrary, lower growth rates by hatchlings feeding on grass shrimp during the first week are probably associated to hatchling small size, and consequently higher difficulty, confirmed from visual observations, in capturing grass shrimp, which are much bigger than mysids.

Growth rates were always significantly higher ($P<0.05$) when feeding grass shrimp, compared to fish, for both live and frozen prey (at similar temperatures), even only after one week of each experiment. This indicates that fish are not an adequate prey for the culture of the early stages of cuttlefish. This is supported by Blanc et al. (1998), who indicates that the diet of young cuttlefish (<8.5 cm ML) captured from the wild was composed essentially of crustaceans (89%), while fish only represented 4.6%.

Growth rates obtained here in all experiments, between 8.6% and 5.1% bw day^{-1}, when using crustaceans are similar to the ones reported by Pascual (1978), of 6.3% and 5.43% bw day^{-1}, DeRusha et al. (1989), of 7.6% bw day^{-1}, at similar temperatures, and by Koueta and Boucaud-Camou (1999) of 7.98% bw day^{-1}, Koueta and Boucaud-Camou (2001) between 7% and 9% bw day^{-1} and Koueta et al. (2002), of 7.5% bw day^{-1}, at lower temperatures averaging 19 °C.

Growth rate is a function of body weight, and therefore, it is expected to decrease with age (and size) of the cuttlefish. The decrease in growth rate with size for this species was reported by many authors (Richard, 1971; Pascual, 1978; Forsythe and Van Heukelem, 1987; Forsythe et al., 1994; Koueta and Boucaud-Camou, 1999; Domingues et al., 2001a, 2002, in press). This fact might help to explain differences in growth rates between the hatchlings, and the larger 30- and 60-day-old juveniles. Results from experiments I to III indicate that grass shrimp are an appropriate prey for the early stages of the life cycle of cuttlefish. Although they are not better than mysids during the first week, they are still good (high growth rates and no mortality) and are the best prey from the second week onwards. Pascual (1978), DeRusha et al. (1989), and Forsythe et al. (1994) have also used *Paleomonetes* sp. to culture *S. officinalis* during their early lives.

The effect of feeding fish to increasingly larger and older cuttlefish can be observed in experiments I to III. Growth rates of cuttlefish aged 1, 30 and 60 days and fed live fish were not different. Nevertheless, Castro and Guerra (1990) reported that there is an increase in the importance of fish and decrease in the importance of crustaceans in the diet of cuttlefish in the wild, as it grows larger. The exact size or age when this change occurs is not clear. Results from the present experiments suggest that at least for growth, until the age of 3 months, the use of fish (both alive and frozen) does not improve growth of larger cuttlefish. Nevertheless, the use of live fish was beneficial for cuttlefish survival as they grew larger. Survival was 73.3% for newly born hatchlings, 91.7% for 30 days juvenile and 100% for 60 days juvenile.

In the experiment IV, frozen prey was used. During this experiment, the same pattern as for the first three experiments, of significantly higher ($P<0.05$) growth for cuttlefish fed grass shrimp compared to fish (Table 4), was observed. Growth rates (Table 4) during the first week for cuttlefish fed frozen shrimp or fish (3.3% and 1.1% bw day^{-1}), respectively,

were much lower than for the following weeks, and than for the average growth rates for the experiment (5.1% and 3.3% bw day^{-1}, respectively). This was due to the lower feeding rates (Table 4) during this first week (10.4% and 7.4% bw day^{-1}, respectively) compared to average feeding rates for the rest of the experiment (12.4% and 11.7% bw day^{-1}, respectively). We believe that this is due to a period of adaptation to the inert food. When results from growth rates during the first week of experiment IV are eliminated, average growth rates for the rest of the experiment are 5.6% and 3.8% bw day^{-1}, when feeding frozen shrimp or fish, respectively. Transition periods from live to frozen prey were also observed by DeRusha et al. (1989), Lee et al. (1991) and Domingues (1999), when culturing *S. officinalis*.

Some authors report differences in growth when feeding live or frozen crustaceans to *S. officinalis* (Pascual, 1978; DeRusha et al., 1989; Lee et al., 1991), while others report no differences (Domingues et al., unpublished results). The similar growth rates using live or frozen prey in this experiment indicate that the freezing process did not affect negatively the growth or survival of the cuttlefish.

Feeding rates in experiment IV (Table 4) were higher (average of 12.4% bw day^{-1}) than the ones obtained by Pascual (1978), at similar temperatures (average of 25 °C). This author obtained feeding rates varying between 7.7% and 9.6% bw day^{-1}. Although food supplied was 20% bw day^{-1}, feeding rates in the experiment were always lower. This was due to the fact that although all shrimp were consumed in each replicate during the experiment, it was not infrequent to find shrimp heads that were not eaten. The experimental design prevented higher feeding rates. If more feeding periods were installed, we believe feeding rates could have reached values close to 40% bw day^{-1} as in previous experiments (Domingues et al., 2001a), or similar (DeRusha et al., 1989; Koueta and Boucaud-Camou, 2001). Nevertheless, Koueta and Boucaud-Camou (2001) and DeRusha et al. (1989) suggest average feeding rates between 15% and 20% bw day^{-1} for this species, while other authors such as Koueta and Boucaud-Camou (1999, 2001) and Forsythe et al. (1994) suggest feeding rates below 20% bw day^{-1}. Because of this, we believe that although feeding rates could have been higher in the present experiment, the one used was not limiting and therefore did not weaken the experimental design.

Food conversions for cuttlefish fed live grass shrimp in experiment IV varied between 36% and 50% (Table 4), and fall within values reported by Pascual (1978), DeRusha et al. (1989) and Koueta and Boucaud-Camou (1999, 2001).

Average food conversions with frozen fish (28%) in experiment IV were significantly lower ($P < 0.05$), compared to when feeding frozen shrimp (41%). The use of frozen fish also promoted lower food conversions than the ones reported by the researchers mentioned above when using crustaceans. Nevertheless, overall feeding rates in experiment IV were similar for both diets (Table 4). This indicates that fish was well accepted and easily captured by the cuttlefish, which we also confirmed by visual observations. Therefore, as shown in Table 4, it was the significantly lower food conversions throughout the experiment that explains the lower growth of cuttlefish fed fish, compared to frozen shrimp. This proves that it is not the difference in the degree of difficulty in catching prey tested that made cuttlefish grow less in the first three experiments, but the quality of the food. Therefore, it is likely that it is the composition of fish that promotes such lower food conversions (and lower growth) compared to shrimp.

Several studies have shown that cephalopods are rich in phospholipids (especially PE and PC), cholesterol and PUFA (Sinanoglou and Miniadis-Meimaroglou, 1998, 2000; Navarro and Villanueva, 2000; Domingues et al., in press). Also, biochemical composition of prey that have been used with success as a food sources for rearing cephalopods shows higher contents in these components (Krzynowek and Panunzio, 1989; Navarro and Villanueva, 2000; Domingues et al., in press). This suggests that cephalopod paralarvae and juvenile require foods rich in cholesterol, phospholipids and polyunsaturated fatty acids (PUFA), and a moderate content in neutral lipids as triglycerides and esterol ester (Navarro and Villanueva, 2000; Koueta et al., 2002).

In the present experiment, we found high level of phospholipids (especially PE) and cholesterol in grass shrimp compared to mysid shrimp and fish fry (Table 5). Besides, fish fry had higher levels of esterol ester (Table 5) which is used as an energy reserve, opposed to the structural role of cholesterol and phospholipids. Considering the profile requirement of cephalopods mentioned before, the lipid class composition suggests that grass shrimp have a higher nutritional quality, compared to the other prey analyzed. Overall composition of grass shrimp is also closer to the one of 30-day-old cuttlefish (Domingues et al., in press) compared to mysids and fish.

Koueta et al. (2002) obtained better growth (9.5% bw day^{-1}) for hatchlings of *S. officinalis* fed mysids enriched with PUFA, compared to non-enriched mysids (7.5% bw day^{-1}). When comparing the PUFA composition from the different prey in the present study, it is noticeable the difference observed in the DHA/EPA ratio, which is similar between the two crustacean species (around 0.7) and clearly higher in fish fry (2.7). These values resemble those obtained in several shrimp species by Krzynowek and Panunzio (1989). DHA and EPA are included in the n-3 highly unsaturated fatty acid (n-3 HUFA) group. DHA plays a multifunctional role in a wide variety of adaptive processes, which occur in cell membranes, whereas the EPA is one of the most important eicosanoids precursors, which are implicated in numerous physiological processes. (Arts et al., 2001; Bell et al., 1986; Sargent et al., 1995). Both fatty acids have showed to be essentials in the early development of marine fish larvae (Watanabe, 1993). According to this, an optimal DHA/EPA ratio could be relevant for the correct development of cuttlefish. Finally, the differences found in the EPA/AA ratio in fish fry (2.1) with respect to crustacean species (around 5) are relevant. Similarly to EPA, arachidonic acid (AA, 20:4n-6) is an important eicosanoid precursor (Sargent et al., 1995) and competes with EPA for the enzymes (cycloxigenase and lipoxigenase) used for eicosanoid production. Thus, variations in the concentrations of AA and EPA could have profound effects for both the spectrum and quantity of eicosanoids production with subsequent alterations in several physiological functions (Mustafa and Srivastava, 1989).

Lipid composition of frozen shrimp indicates a reduction on polar lipid contents (namely on PE percentages) and an increase on FFA percentages when compared with live shrimp. Both alterations used can be associated to oxidation processes. Therefore, the nutritional quality of lipids from frozen shrimp should be lower than that of lipids from live prey.

Carotenoids have been described as potent antioxidants and are related with a correct development of several species (Liebler, 1993)‘. In the present experiment, higher content of carotenoids has been described in crustacean species compared with fish fry. This fact could also have contributed to the differential effects on growth and survival observed when

cuttlefish were fed crustacean species or fish fry. Nevertheless, further studies are necessary to access the possible influence of carotenoids on growth and survival of young cuttlefish.

Finally, to understand the real importance of lipids, particularly of polar lipids, for cuttlefish, biochemical composition of amino acids and carotenoids, among others, as well as protein digestibility of these prey must be studied.

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