Aquaculture International **11:** 397–410, 2003.

Effects of feeding live or frozen prey on growth, survival and the life cycle of the cuttlefish, *Sepia officinalis* (Linnaeus, 1758)

PEDRO DOMINGUES[1,*], ANTÓNIO SYKES[1],
ANNE SOMMERFIELD[2] and JOSÉ P. ANDRADE[1]
*[1]Faculdade de Ciências do Mar e do Ambiente, CCMar – Universidade do Algarve, Campus de Gambelas – 8000-810 Faro, Portugal; [2]University Cork College, Cork, Republic of Ireland; *Author for correspondence (e-mail: pdoming@ualg.pt)*

Received 3 September 2002; accepted in revised form 29 April 2003

Key words: Cephalopod, Cuttlefish, Food conversions, Frozen shrimp, Life cycle, Live shrimp

Abstract. The effects of feeding live or frozen grass shrimp (*Palaemonetes varians*) to the cuttlefish, *Sepia officinalis*, were determined in two experiments. During Experiment I, two populations of 30 cuttlefish (aged 90 days old) were fed either live or frozen grass shrimp. Cuttlefish fed live shrimp grew larger, matured earlier, had a shorter life cycle (255 days) than the ones fed frozen shrimp (282 days), and had lower mortality. Females from the group fed frozen shrimp matured a month later but were significantly larger, 130.9 ± 38.5 g, compared to 74.2 ± 16.0 g, laid larger eggs, 0.47 ± 0.11 g, compared to 0.28 ± 0.10 g, and had higher individual fecundity (411 eggs female^{-1}, compared to 150 eggs female^{-1}). Newly born hatchlings from both groups had similar weights. During Experiment II, six replicates of 15 cuttlefish (50 days old) were used, three for each of the two diets tested. The exact same amount of live or frozen shrimp was provided to both populations twice a day. No differences in growth and feeding rates or food conversions were found at the end of the experiment. During the first week, cuttlefish fed frozen shrimp grew larger, and had higher conversion rates, compared to the ones fed live shrimp. Mortality was higher for the group fed live shrimp (36.6%) in Experiment II, mainly occurring during the last week. Mortality for cuttlefish fed frozen shrimp in Experiment II was 2.2%. Results obtained here indicate that freezing the grass shrimp only had a negative effect on the survival of *S. officinalis* in Experiment I.

Introduction

The European cuttlefish, *Sepia officinalis* belongs to the family Sepiidae. About 100 species of cuttlefish have been found around the world (Okutani 1990). From these, *S. officinalis*, is one of the most well known, and the most easily cultured (Forsythe et al. 1994). This species has been grown in laboratories and public aquaria around the world (Boletzky 1983; Clarke et al. 1989, Domingues 1999) and more than two or three consecutive generations have been cultured throughout the years (Richard 1966; Pascual 1978; Boletzky 1979; Toll and Strain 1988; Forsythe et al. 1994; Lee et al. 1998; Domingues et al. 2001b).

Cuttlefish (*S. officinalis*) hatchlings are born as miniature replicas of adults, and have similar basic behavior as adults, namely a marked benthic mode of life (Warnke 1994). During the first few weeks of their life, cuttlefish have to be fed live

prey, usually mysid shrimp (Richard 1975; Forsythe et al. 1994; Domingues 1999; Domingues et al. 2001a). Afterwards, they will accept dead food, such as frozen shrimp, fish or crabs (DeRusha et al. 1989; Forsythe et al. 1991; Domingues et al. 2001a). Some authors have cultured this species making this transition to dead food (Pascual 1978; Forsythe et al. 1994), while others fed live prey throughout the life cycle (Domingues et al. 2001a, 2001b, 2002).

Therefore, this is one of the most promising species for large-scale culture. It has been cultured, with yields of up to 1000 individuals per generation, using live mysids (*Paramysis nouvelli*) and live grass shrimp (*Palaemonetes varians*) throughout the life cycle (Domingues et al. 2001b, 2002) with excellent results. Nevertheless, to achieve large-scale culture the dependence on live wild shrimp caught in the ponds surrounding the culture facility has to be broken. Freezing prey to be stocked and used later, when necessary, is a good way to assure larger and continuous productions.

During the present study, two experiments were conducted. The first experiment consisted of feeding either live or frozen grass shrimp (*P. varians*) to two groups of *S. officinalis* from 3 months old to the end of their life, in order to determine the effect on several aspects of the life cycle such as growth, survival, fecundity and egg viability. A second short experiment was conducted to determine the effects of feeding the same live or frozen grass shrimp to younger (50 day old) cuttlefish. Growth and feeding rates, and food conversion were measured in order to determine if the freezing procedure was having an effect on the quality of the diet.

Material and methods

Experiment I

Two groups of 30 cuttlefish aged 90 days old were used in this experiment. Cuttlefish were cultured from hatchlings to 90 days old on live mysids (*P. nouvelli*) during the first 15 days of life, and with live grass shrimp until 3 months old. They were part of a third generation cultured in captivity in the laboratory. At the start of the experiment (90 days old), cuttlefish to be fed live or frozen shrimp weighed 17.4 ± 6.0 and 18.9 ± 6.0 g, respectively, and no significant difference in weight ($p > 0.05$) was found (Zar, 1984). The 30 cuttlefish from each group were placed in two fibreglass circular tanks 1 m in diameter, and 40 cm depth (volume of 250–300 l). These tanks were part of a previously described flow-through culture system (Domingues et al. 2001a, 2001b). Before reaching the culture tanks, water passed by a UV filter to improve water quality. Water flow was $120\,l\,h^{-1}$, and lights were on 16 h a day. Temperature at the start of the experiment, at the end of autumn was of 19.4 °C, but quickly decreased to 13.4 °C during the second week, and to lower values (< 12 °C) in the third week. Afterwards, two electric heaters were placed in each tank to raise water temperature, which varied between 18 and 19 °C during the rest of the experiment. Dissolved oxygen in the tanks varied between 60 and 75%.

Water temperature was measured daily, while salinity and dissolved oxygen were measured on a weekly basis. Student's *t*-tests were also performed at every weighing period, to determine differences in weight between the two groups.

The live and frozen shrimp used were captured in the ponds surrounding the field station. They were captured using a handheld net (2 mm mesh size) with an opening of 1.5 m. Fishing was carried out on the sandy bottom, close to shore. Part of the shrimp captured was fed to the cuttlefish, while the remainder was frozen ($-20\,^{\circ}$C) and used within 3 days. Since the freezing process might alter, by oxidation, the levels of lipid in the diets, we compared lipid composition of the live grass shrimp (preserved in liquid nitrogen) with composition of similar shrimp frozen and preserved for a month at $-20\,^{\circ}$C. Results indicated that there was no significant oxidation (Domingues, unpublished data), and therefore the frozen shrimp used here, within 3 days of freezing, should still have high levels of lipids.

Both groups were fed the same amount of food, which varied between 25 and 30% of their body weight (BW) per day (%BW day^{-1}). Food was supplied twice a day, at 10:00 and 15:00 h, respectively. Frozen shrimp was placed in saltwater for 1 min to promote a slight defrost which allowed it to sink immediately when placed in the culture tanks. It was left in the tanks for 2 h, and then netted out, to prevent deterioration of water quality. No acclimation period was necessary, since frozen shrimp were well accepted by cuttlefish of this age. Both groups were weighed every 7 days during the experiment, except where indicated.

Cuttlefish were weighed until egg laying started. After this, animals were not weighed to avoid further stressing. Mean weight was used to calculate the mean instantaneous growth rate (IGR) (%BW day^{-1}) $= ((\ln \mathrm{W2} - \ln \mathrm{W1})/t \times 100)$, where W2 and W1 are the final and initial weight of the cuttlefish, respectively, ln the natural logarithm and t the number of days of the time period. Mortality rates were determined at every weighing interval. When egg laying started, eggs were collected and counted every day, and about 50% of them were weighed, to determine differences between groups. Eggs from each population were placed in different baskets. Duration of egg laying period and embryonic development time were determined. Hatchlings from each batch of eggs were counted, and about 30% of them were weighed to determine any differences in fresh weight between the treatments. Hatching percentage was also determined for each group. After egg laying started, every dead cuttlefish in each group was weighed and sex determination was performed. This allowed determination of number of females reproducing, and therefore average fecundity in each group. Female cuttlefish die after (or during) spawning, while males can live a lot longer. Since the culture tanks were necessary for further experiments, the experiment was ended when the last female in each group had died.

Experiment II

The same two diets used in Experiment I (live and frozen grass shrimp) were used in this experiment. Three replicates of 15 cuttlefish (50 days old) were used for each

diet tested. These cuttlefish were part of a fourth generation (F4) cultured in the research facility. Before the start of the experiment, cuttlefish in each group were weighed and ANOVA's (Zar 1984) were performed to assure that no significant differences were found between the six replicates.

A flow-through system consisting of 12 plastic rectangular tanks (38 cm × 28.5 cm) was used. Water depth was 12 cm, and volume of the tanks was about 10 l. Water flow was $10\,l\,h^{-1}$ and the light cycle was the same as for the previous experiment.

Cuttlefish in each replicate were fed 20%BW day^{-1}. Food was weighed and presented equally, three times per day (9:00, 12:00 and 16:00). To ensure that cuttlefish were fed the same amount of food each time, the groups eating frozen shrimp were fed first. After 1 h, food remains were netted out and weighed, and food eaten was determined. The same biomass of the frozen food eaten was then given to the groups eating live shrimp. Food eaten was registered for each feeding period. Mortality rates were determined for each weighing interval. At the start of the experiment, cuttlefish fed live and frozen shrimp weighed 5.5 ± 0.8 and 5.6 ± 0.9 g, respectively, and were not significantly different ($p = 0.456$; $t = 0.749$). Average temperatures were of 23.9, 24.8, 25.0 and 24.9 °C during the 4 weeks of the experiment, respectively. Salinity varied between 36 and 37 ppt, and dissolved oxygen varied between 70 and 80% in all tanks, during the experiment. Water temperature was measured daily, while salinity and dissolved oxygen were measured on a weekly basis. The experiment lasted for 4 weeks.

Cuttlefish were weighed every 7 days and the data were used to calculate: (1) IGR; (2) feeding rate (FR) (% BW 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); (3) food conversion (FC) = (W2−W1)/FI, where W2−W1 is the weight gained by the cuttlefish during the time period.

After every weighing period, statistical analysis was performed to determine differences in weight between groups. ANOVAs (Zar 1984) were performed between the three replicates of each group, and if no significant differences were found between the three replicates, all cuttlefish in those groups fed the same diet were gathered, and a t-test was performed to compare differences in weight between cuttlefish fed the two diets. Student's t-tests were performed to compare growth and feeding rates and food conversions between the two groups.

Results

Experiment I

Up until the start of egg laying, only four cuttlefish fed live shrimp died (13.3% mortality). A higher mortality rate was observed for cuttlefish fed frozen shrimp (36.6%) over the same period. The high mortality in this group can be attributed to disease (possibly bacterial infection), since all animals had skin ulcerations, some exposing the cuttlebone, while none of the group fed live shrimp had them.

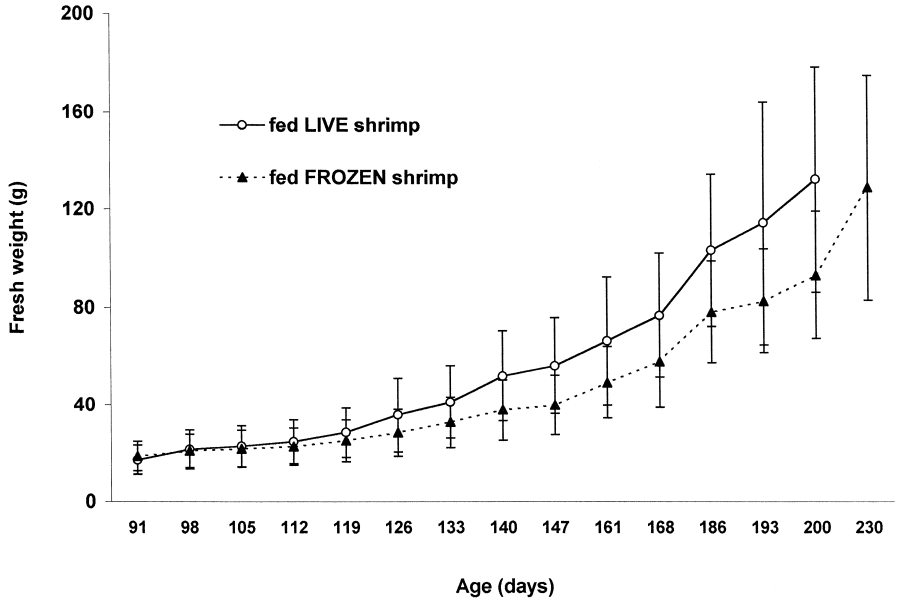

Figure 1. Growth of two groups of cuttlefish (*S. officinalis*) fed either live or frozen grass shrimp (*P. varians*) from 90 days old until the end of the life cycle during Experiment I. Bars indicate standard deviations.

Nevertheless mortality in this group remained low until about 150 days old (10%), only increasing to the final value reported here during the following 3 weeks.

Figure 1 shows the growth of both populations. At 126 days old, cuttlefish fed live shrimp weighed 35 ± 8.0 g, and were significantly larger ($p = 0.02$) than the ones fed frozen shrimp (28.5 ± 9.7 g). From that point onwards, until the start egg laying at 204 days old, cuttlefish fed live shrimp were always larger ($p < 0.05$). Cuttlefish fed frozen shrimp matured about 30 days later, and only started laying eggs at 234 days old. At the weighing period prior to the start of egg laying, cuttlefish fed live or frozen shrimp had similar weights ($p > 0.05$) of 132.6 ± 46.0 and 129.3 ± 46 g, respectively.

Figure 2 shows growth rates (% BW day^{-1}) and water temperature for every weighing interval, until egg laying started in both groups. Growth rates were much higher for the group fed live shrimp during the first 2 weeks (6.7 and 3.8%BW day^{-1}) compared to the ones fed frozen shrimp (1.5 and 0.6%BW day^{-1}). Between 105 and 112 days old, water temperatures were very low, which accounted for the low growth rates in both groups. Until about 150 days old, cuttlefish fed live shrimp had higher growth rates. From that period onwards, no clear pattern appeared, but growth rates were very low for both groups (< 3%BW day^{-1}).

Table 1 shows the comparison between several aspects of the life cycle of both experiments. Life cycle of cuttlefish fed frozen shrimp was longer (282 days,

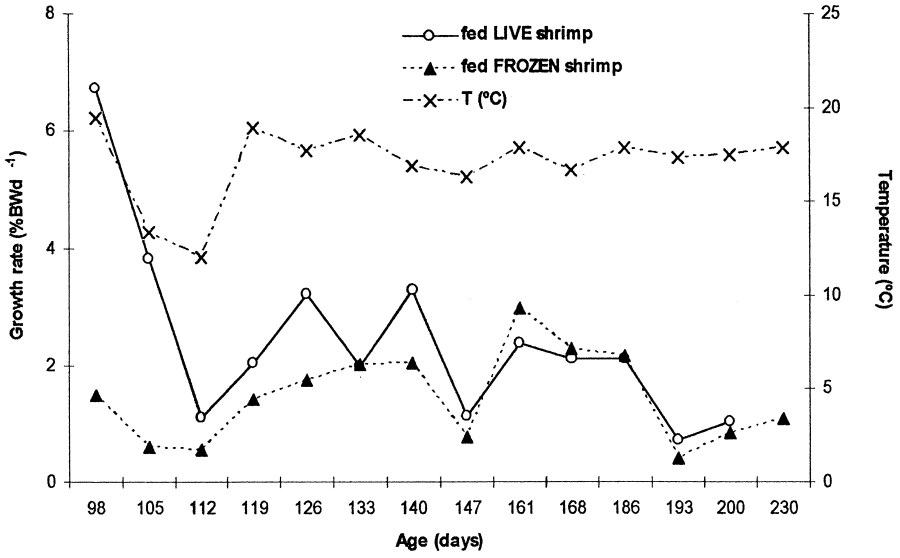

Figure 2. Growth rates (% body weight day^{-1}) of two groups of cuttlefish (*S. officinalis*) fed either live or frozen grass shrimp (*P. varians*) from 90 days old until the end of the life cycle during Experiment I.

Table 1. Results for the two life cycles of *S. officinalis* cultured in the laboratory during Experiment I. One population was fed live grass shrimp (*P. varians*), while the second population was fed the same shrimp that had been previously frozen.

	Fed live shrimp	Fed frozen shrimp
Duration of life cycle (days)	255	282
Duration of spawning period (days)	55	52
Eggs laid (*n*)	1800	3700
Eggs (*n*)/female	150	411
Eggs sampled (*n*)	900	1800
Mean weight eggs (g)	0.28 ± 0.10	0.47 ± 0.11
Hatchlings sampled (*n*)	400	960
Mean weight hatchlings (g)	0.100 ± 0.016	0.099 ± 0.022
Mean weight females (g); (*n*)	72.4 ± 16.0 (12)	130.9 ± 38.5 (9)
Mean weight males (g); (*n*)	211.6 ± 93.4 (13)	221.4 ± 82.1 (9)
Incubation temperature; time to hatch	16.6 ± 1.2 °C; 48 days	18.8 ± 0.8 °C; 34 days
Hatching percentage	33%	85%

compared to 255 days). Females from the group fed frozen shrimp matured about a month later, but were larger ($p = 0.0000$) at death, compared to the ones fed live shrimp. They laid larger eggs ($p = 0.0000$), but hatchlings from both groups had similar sizes ($p = 0.9242$). For both groups, males were larger than females at death ($p = 0.0000$ and 0.0042, for cuttlefish fed live and frozen shrimp, respectively). Males from both groups had similar weights at death ($p = 0.4003$). Individual fecundity and hatching rates (egg quality) were almost three times higher for females fed frozen shrimp. Since egg laying started about a month later for cuttlefish fed

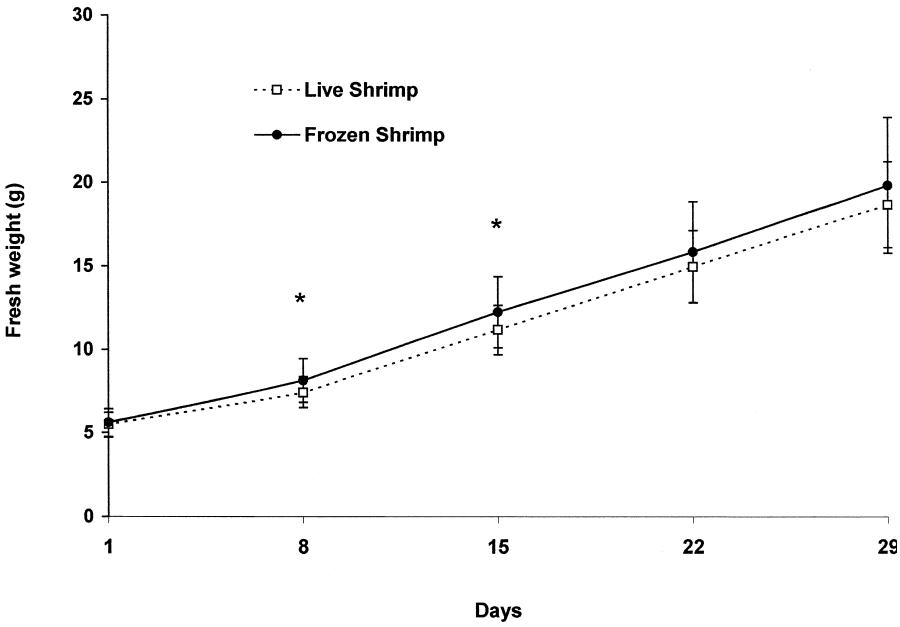

Figure 3. Growth of two groups of cuttlefish (*S. officinalis*) fed either live or frozen grass shrimp (*P. varians*). Experiment II started at 50 days old, and lasted 4 weeks. A total of six replicates (three per diet) of 15 cuttlefish each were used. The '*' indicates that the values were significantly different ($p < 0.05$). Bars indicate standard deviations.

frozen shrimp, the water temperature was already about 2 °C higher compared to when the ones fed live shrimp started laying eggs. Egg development time took 2 weeks less at 18.8 °C (group fed frozen shrimp) than at 16.6 °C (group fed live shrimp).

Experiment II

Figure 3 shows growth for both groups. Although weight at the start of the experiment was not different, cuttlefish fed frozen shrimp were significantly larger (8.2 ± 1.3 g compared to 7.5 ± 0.9 g) during the second week ($p = 0.037$; $t = 2.978$) and the third week ($p = 0.007$; $t = 2.764$), weighing 12.2 ± 2.1 g, compared to 11.2 ± 1.5 g for the ones fed live shrimp. However after week 3 and until the end of the experiment, weights of both groups were not significantly different ($p > 0.05$). At the end of the experiment, cuttlefish fed frozen and live shrimp weighed 19.8 ± 4.1 and 18.7 ± 2.6 g, respectively, and were not significantly different ($p = 0.142$; $t = 1.482$).

Figure 4 shows the growth rates (%BW day^{-1}) for the two groups. Growth rates varied between 3 and 6%BW day^{-1} for both groups, being higher for the first half of the experiment, and then decreasing gradually. Only during the first week were

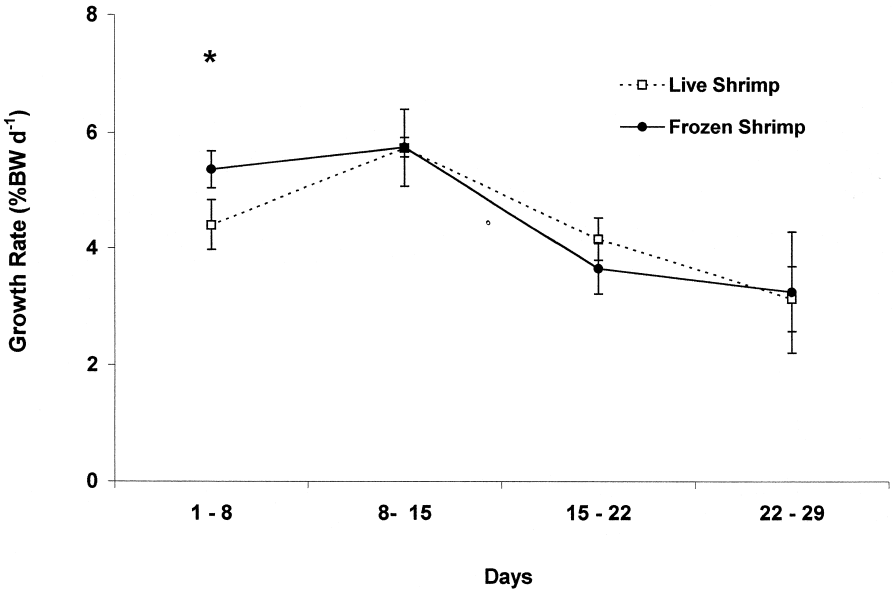

Figure 4. Growth rates (% body weight day^{-1}) for cuttlefish (*S. officinalis*) fed either live or frozen grass shrimp (*P. varians*). Experiment II started at 50 days old, and lasted 4 weeks. A total of six replicates (three per diet) of 15 cuttlefish each were used. The '*' indicates that the values were significantly different ($p < 0.05$). Bars indicate standard deviations.

growth rates for cuttlefish fed frozen shrimp (5.4 ± 0.3%BW day^{-1}) higher ($p = 0.038$; $t = 3.058$) than for the ones fed live shrimp (4.4 ± 0.4%BW day^{-1}). For the next 3 weeks until the end of the experiment, there were no significant differences ($p > 0.05$) in growth rates between the two groups.

Figure 5 shows feeding rates for both groups. Feeding rates decreased consistently from the start of the experiment, and were always higher for the cuttlefish fed live shrimp, although significant differences existed only during the first week ($p = 0.009$; $t = 4.787$). Feeding rates varied between 14.1 ± 0.2 and 8.9 ± 0.2%BW day^{-1} for cuttlefish fed live shrimp, and between 13.1 ± 0.3 and 8.3 ± 0.3%BW day^{-1} for cuttlefish fed frozen shrimp.

Figure 6 shows food conversions for the two groups. In accordance with the feeding rates, cuttlefish fed frozen shrimp (0.40 ± 0.01) had significantly higher food conversions ($p = 0.009$; $t = 4.794$) during the first week, compared to the ones fed live shrimp (0.31 ± 0.03). Food conversions for cuttlefish fed live shrimp varied between 31 and 46%, while for the ones fed frozen shrimp it varied between 34 and 49%.

Mortality rates were very low in the three replicates fed frozen shrimp. Only during the last week of the experiment was there one cuttlefish dead (2.2% mortality rate). For the groups fed live shrimp, one cuttlefish died between the second and third week, while during the fourth week nine more cuttlefish died (three in each replicate).

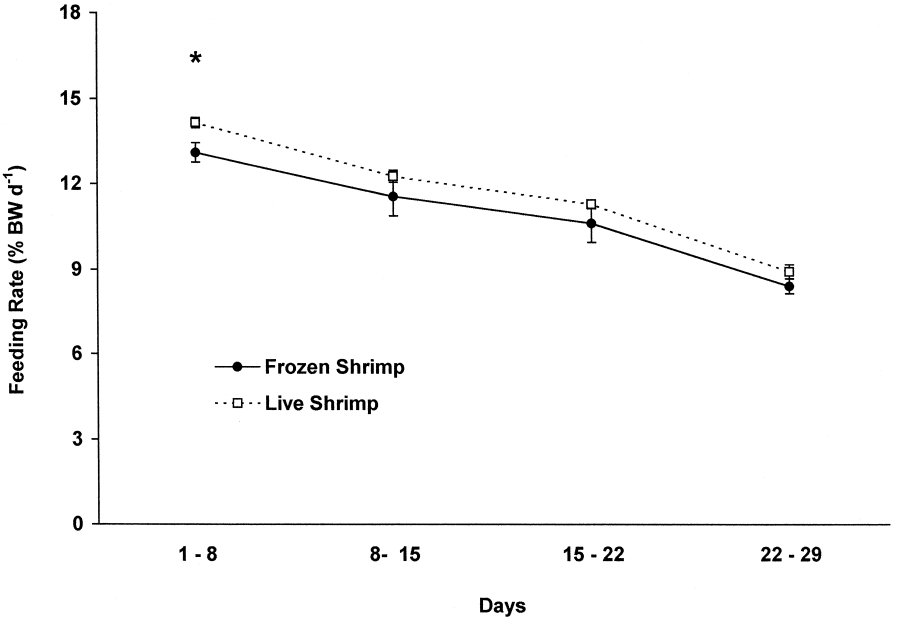

Figure 5. Feeding rates (% body weight day^{-1}) for cuttlefish (*S. officinalis*) fed either live or frozen grass shrimp (*P. varians*). Experiment II started at 50 days old, and lasted 4 weeks. A total of six replicates (three per diet) of 15 cuttlefish each were used. The '*' indicates that the values were significantly different ($p < 0.05$). Bars indicate standard deviations.

Discussion

When comparing the effects of feeding live or frozen shrimp to cuttlefish, there is a strong indication that freezing prey did not have negative effects in many relevant aspects of the life cycle. The only important exception was the higher mortality in Experiment I. Results for duration of the life cycle, weight of both males, and especially females, individual fecundity and more importantly, egg quality (hatching rate), were better when using frozen shrimp. This finding is supported by Experiment II, where there were no significant differences ($p > 0.05$) in growth between both groups at the end of the experiment. Furthermore, food conversions were even higher for the group fed frozen shrimp during the first week and, contrary to what happened in Experiment I, cuttlefish mortality was even lower.

The duration of the life cycle of cuttlefish fed live shrimp (255 days) cultured at average temperatures of 18 °C was similar to the one for the previous generation (F2) reported by Domingues et al. (2002), of 260 days, for cuttlefish cultured at average temperatures of 15 °C. The life cycle of population fed frozen shrimp was considerably longer, reaching 282 days, being the longest cultured by this research group up to the present stage. From the four generations cultured until now, including two cycles (winter and summer cycles) for both the third and fourth generation, no cuttlefish reached 10 months old. Life cycles obtained in the Algarve facility are shorter that the

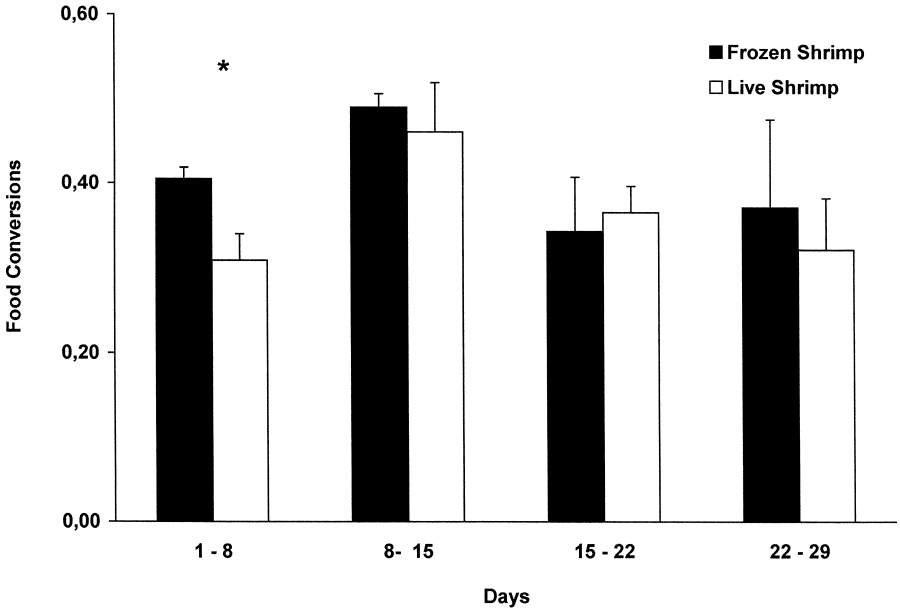

Figure 6. Food conversions for cuttlefish (*S. officinalis*) fed either live or frozen grass shrimp (*P. varians*). Experiment II started at 50 days old, and lasted 4 weeks. A total of six replicates (three per diet) of 15 cuttlefish each were used. The '*' indicates that the values were significantly different ($p < 0.05$). Bars indicate standard deviations.

ones reported by other authors. Forsythe et al. (1994) reported average life cycles of 11, 14 and 17 months for cuttlefish cultured at temperatures varying between 20 and 24 °C. Richard (1971) reported life cycles of over a year, at 15 °C. Pascual (1978) and Le Goeff and Daguzan (1991) also reported longer life cycles for this species.

The generally lower growth obtained for the population fed frozen shrimp, especially at the start of Experiment I, could not be explained by the acclimation period, since cuttlefish ate almost all frozen shrimp provided, from the second feeding period onwards. Nevertheless, the necessity of removing uneaten remains 2 h after feeding was a disadvantage, since the batch receiving live food ate all the shrimp provided over the 24 h period. However the other group only ate part of the frozen shrimp (uneaten frozen shrimp was frequently removed from the tanks after each feeding period). This is one of the most important ways of maintaining water quality in the culture system, as the frozen shrimp are potential vehicles for infection (Loi and Tublitz 1998).

Considering that cuttlefish used in Experiment I were already 3 months old, growth rates obtained here were similar to the ones reported by Domingues et al. (2002), which were close to 3%BW day^{-1} for cuttlefish of similar age and size. The only exception was the first week of the experiment for cuttlefish fed live shrimp, which grew at almost 7%BW day^{-1}. Growth rates below or around 2%BW day^{-1} for the last part of the life cycle were also similar to the ones reported by Richard (1975) and Domingues et al. (2002).

Koueta and Boucaud-Camou (1999) compared feeding rates of young *S. officinalis* fed either live prey (mysids and small shrimp) or dead prey (frozen mysids) and found no differences in feeding rates (11.76 and 10.84%BW day^{-1}, for cuttlefish fed live and frozen prey, respectively). Feeding rates obtained in Experiment II were higher than those reported by these authors being over 13%BW day^{-1}, during the first part of the experiment, for both groups. However feeding rates close to 9%BW day^{-1}, towards the end of the Experiment II were slightly lower than the ones reported by Koueta and Boucaud-Camou (1999). Similarly, no significant differences in feeding rates (except for week 1) for the live and frozen shrimp were found in the present study. In contrast Koueta and Boucaud-Camou (1999) reported higher growth rates (7.3%BW day^{-1}) for cuttlefish fed live prey, compared to the ones fed frozen mysids (4.6%BW day^{-1}). DeRusha et al. (1989) also reported that *S. officinalis* (30–160 g in wet weight) fed frozen diets grew 5–8% less compared to others fed live prey. This was not observed in Experiment II, where food supplied was the same for both groups. Nevertheless, in Experiment I, better growth for cuttlefish fed live food was observed, probably due to the different methodology used, which resulted in a lower food consumption by cuttlefish fed frozen shrimp in Experiment I.

The results obtained in Experiment I regarding the life cycle of the population fed frozen shrimp can possibly be explained by one important factor that occurred during the experiment. As mentioned before, disease (possibly bacterial infection) occurred in the group fed frozen shrimp, and this may have been caused by bacteria that were present in dead food. At about 150 days old, every cuttlefish in the group fed frozen shrimp started to develop skin injuries, in many cases exposing the cuttlebone. This debilitated the cuttlefish, and they started to eat less and their growth rate decreased. About 25% of the population died during that month, but eventually they recovered (closing even the open wounds in the flesh). Nevertheless, this fact most likely slowed their growth and maturation, and therefore their life cycle. This might have been an important factor in explaining the longer life cycle and higher weight (130.9 ± 38.5 g) attained by females fed frozen shrimp, when compared to the ones fed live shrimp (72.4 ± 16.0 g), which matured earlier and started spawning sooner. This could also explain the much lower fecundity and egg size from the live shrimp group.

Individual fecundity (411 eggs female^{-1}) from females fed frozen shrimp was the highest obtained, in all four generations cultured in captivity. This value is even higher than the upper limit reported for females from the Ria Formosa by Villa (1998), with individual fecundity varying between 98 and 385 eggs female^{-1}. Fecundity from this group falls in the range reported by Mangold-Wirz (1963), between 200 and 500 eggs female^{-1}, and Richard (1971), between 150 and 500 eggs female^{-1} for this species. Nevertheless, they are much lower than fecundity reported by Forsythe et al. (1994) of over 3000 eggs female^{-1} and Hanley et al. (1998) of 2000 eggs female^{-1}.

Egg size is directly related to female size (Boletzky 1983). Therefore, it is natural that eggs from females fed frozen shrimp (almost twice as large than females fed live shrimp) were significantly larger ($p = 0.0000$). Nevertheless, hatchlings from both groups were similar in fresh weight at birth ($p = 0.9242$). Egg development

from females fed frozen shrimp, taking place at 18.8 °C, was much faster, taking 2 weeks less compared to the ones from females fed live shrimp that developed at average temperatures of 16.6 °C. A faster embryonic development time at higher temperatures was also reported by Choe (1966), Boletzky (1974), Bouchaud and Daguzan (1989) and Caverivière et al. (1999), and explains the much shorter development time for eggs from the population fed frozen shrimp. Larger hatchlings from the eggs from females fed frozen shrimp might have been expected, since these were almost twice as big, compared to the ones from females fed live shrimp (0.47 ± 0.11 and 0.28 ± 0.10 g, respectively). However, Bouchaud (1991) indicates that hatchlings born from eggs kept at lower temperatures are larger. In addition Bouchaud and Daguzan (1990) reported that yolk absorption is faster but lower at higher temperatures resulting in smaller hatchlings. The fact that embryonic development was almost 30% faster for the larger eggs from the frozen shrimp group explains why hatchlings from these larger eggs were not bigger.

Results from these experiments indicate that frozen shrimp are as good as live shrimp to culture *S. officinalis* even although total protein contents of the diet were probably affected (leaching) by the freezing procedure. Also, lipid contents could have been modified by oxidation processes. Koueta et al. (2002), as well as Navarro and Villanueva (2000) have suggested the importance of polyunsaturated fatty acids (PUFA) for *S. officinalis*, especially during the early stages of the life cycle where components such as DHA and EPA are very important for the formation of the eye, membranes and nervous system (Uauy et al. 2000). Research on this subject is also being conducted, after much lower growth rates were noticed when feeding young cuttlefish with live or frozen fish fry compared to live or frozen grass shrimp (Domingues unpublished data).

The possibility of using frozen shrimp is a great advantage if large numbers of cuttlefish are to be produced, as large quantities of food can be stocked to be used when live food is not available. This allows the production of *S. officinalis* at inland sites, far away from the sea, and it also ends the dependence on live shrimp, their holding tanks and the feeds to maintain them. These factors can significantly reduce production costs of the cuttlefish.

Acknowledgements

Pedro Domingues would like to thank the Fundação para a Ciência e Tecnologia and the program PRAXIS XXI (BPD 22057/99) from the Portuguese government, for the financial support for this research.

References

Boletzky S. 1974. Elevage de Céphalopodes en aquarium. Vie Milieu 24: 309–340.

Boletzky S. 1979. Growth and life-span of *Sepia officinalis* under artificial conditions. Rapport Communitaire Internationel Mer Méditeranee 25/26 (10): 10.

Boletzky S. 1983. *Sepia officinalis*. In: Boyle P.R. (ed) Cephalopod Life Cycles. Vol. I. Academic Press, London, pp. 31–52.

Bouchaud O. 1991. Energy consumption of the cuttlefish *Sepia officinalis* L. (Mollusca: Cephalopoda) during embryonic development, preliminary results. Bull. Mar. Sci. 49 (1–2): 333–340.

Bouchaud O. and Daguzan J. 1989. Etude de dévelopement de l'oeuf de *Sepia officinalis* L. (Céphalopode, Sepioidea) en conditions expérimentales. Haliotis 19: 189–200.

Bouchaud O. and Daguzan J. 1990. Etude expérimentale de l'influence de la température sur le déroulement du développement embryonnaire de la Seiche *Sepia officinalis* L. (Céphalopode, Sepioidae). Cahiers Biol. Mar. 31: 131–145.

Caverivière A., Domain F. and Diallo A. 1999. Observations of the influence of temperature on the length of embryonic development in *Octopus vulgaris* (Senegal). Aquat. Living Res. 12: 151–154.

Clarke A., Rodhouse P.A., Holmes L.J. and Pascoe P.J. 1989. Growth rate and nucleic acid ratio in cultured cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda). J. Exp. Mar. Biol. Ecol. 133: 229–240.

Choe S. 1966. On the eggs, habits of the fry and growth of some cephalopoda. Bull. Mar. Sci. 16: 330–348.

DeRusha R.H., Forsythe J.W., DiMarco F.P. and Hanlon R.T. 1989. Alternative diets for maintaining and rearing cephalopods in captivity. Lab. Anim. Sci. (American Association for Laboratory Animal Science) 39: 306–312.

Domingues P.M. 1999. Development of alternative diets for the mass culture of the European cuttlefish *Sepia officinalis*. Ph.D. Thesis.University of the Algarve, 95 pp.

Domingues P.M., Kingston T., Sykes A. and Andrade J.P. 2001a. Growth of young cuttlefish, *Sepia officinalis* (Linnaeus, 1758) at the upper end of the biological distribution temperature range. Aquacul. Res. 32: 923–930.

Domingues P.M., Sykes A. and Andrade J.P. 2001b. The use of artemia or mysids as food for hatchlings of the cuttlefish *Sepia officinalis* Linnaeus, 1758; effects on growth and survival throughout the life cycle. Aquacult. Int.: 9: 319–331.

Domingues P.M., Sykes A. and Andrade J.P. 2002. The effects of temperature in the life cycle of two consecutive generations of the cuttlefish *Sepia officinalis* (Linnaeus, 1758), cultured in the Algarve (South Portugal). Aquacult. Int.: 10: 207–220.

Forsythe J.W., Hanlon R.T. and DeRusha R.H. 1991. Pilot large-scale culture of *Sepia* in biomedical research. In: Boucaud-Camou E. (ed) The Cuttlefish. Centre de publications de l'Université de Caen, pp. 313–323.

Forsythe J.W., DeRusha R.H. and Hanlon R.T. 1994. Growth, reproduction and life span of *Sepia officinalis* (Cephalopoda: Mollusca) cultured through seven consecutive generations. J. Zool. Lond. 233: 175–192.

Hanley J.S., Smolowitz R., Bullis R.A., Mebane W.N., Gabr H.R. and Hanlon R.T. 1998. Modified laboratory culture techniques for the European cuttlefish *Sepia officinalis*. Biol. Bull. Mar. Biol. Lab. Woods Hole 195: 223–225.

Koueta N. and Boucaud-Camou E. 1999. Food intake and growth in reared early juvenile cuttlefish *Sepia officinalis* L. (Mollusca: Cephalopoda). J. Exp. Mar. Biol. Ecol. 240: 93–109.

Koueta N., Boucaud-Camou E. and Noel B. 2002. Effects of enriched natural diet on survival and growth of juvenile cuttlefish *Sepia officinalis* L. Aquaculture 203: 293–310.

Lee P.G. 1994. Nutrition of cephalopods: fuelling the system. Mar. Freshwater Behav. Physiol. 25: 35–51.

Lee P.G., Turk P.E., Forsythe J.W. and DiMarco F.P. 1998. Cephalopod culture: physiological, behavioral and environmental requirements. Suisanzoshoku 46: 417–422.

Le Goeff R. and Daguzan J. 1991. Growth and life cycles of the cuttlefish *Sepia officinalis* L. (Mollusca: Cephalopoda) in South Brittany (France). Bull. Mar. Sci. 49: 341–348.

Loi P.K. and Tublitz N.J. 1998. Long term rearing of cuttlefish in a small scale facility. Aquarium Sci. Conserv. 2: 1–9.

Mangold-Wirz K. 1963. Biologie des céphalopodes benthiques et nectoniques de la Mer Catalane. Vie et Milieu 13: 285.

Navarro J.C. and Villanueva R. 2000. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. Aquaculture 183: 161–177.

Okutani T. 1990. Squids, cuttlefish and octopuses. Mar. Behav. Physiol. 18: 1–17.

Pascual E. 1978. Crecimiento y alimentación de tres generaciones de *Sepia officinalis* en cultivo. Investigación Pesquera 42: 421–442.

Richard A. 1966. La temperature, facteur externe essentiel de croissance pour le céphalopode *Sepia officinalis* L. C.r. hebd. Séanc. Acad. Sci. Paris 263: 1138–1141.

Richard A. 1971. Contribuition à l'étude expérimentale de la croissance et de la maturation sexuelle de *Sepia officinalis* L. (Mollusque, Céphalopode). Thèse no 248:Univ. Lille, 264 pp.

Richard A. 1975. L'elevage de la seiche (*Sepia officinalis* L., Mollusque, Céphalopode). In: Proceedings of the 10th European Symposium on Marine Biology, Ostend, Belgium. Vol. 1, pp. 359–380.

Toll R.B. and Strain C.H. 1988. Freshwater and terrestrial food organisms as an alternative diet for laboratory culture of cephalopods. Malacologia 29: 195–200.

Uauy R., Mena P. and Rojas C. 2000. Essential fatty acids in early life: structural and functional role. Proce. Nutr. Soc. 59: 3–15.

Villa H. 1998. Estudo de alguns aspectos da biologia reprodutiva da espécie *Sepia officinalis* (Linnaeus, 1758) na Ria Formosa. MSc Thesis.University of Algarve, 82 pp.

Warnke K. 1994. Some aspects of social interaction during feeding in *Sepia officinalis* (Mollusca: Cephalopoda) hatched and reared in the laboratory. Vie Millieu 44 (2): 125–131.

Zar J.H. 1984. In: McElroy W. and Swanson P. (ed) Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ, 619 pp.