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EARLIER ACCEPTANCE OF FROZEN PREY BY JUVENILE CUTTLEFISH *SEPIA OFFICINALIS* IN EXPERIMENTAL REARING: EFFECT OF PREVIOUS ENRICHED NATURAL DIET

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CULTURE
CUTTLEFISH
FISH OIL
GROWTH
PUFA
SURVIVAL

ABSTRACT. – From among hatchlings, juvenile cuttlefish *Sepia officinalis* of 200 mg were divided into two groups fed with two different diets: natural live prey and PUFA (polyunsaturated fatty acids) enriched natural live prey. After 10 days of rearing, juvenile cuttlefish previously fed on the enriched diet easily accepted natural frozen prey and their survival was better, while juvenile cuttlefish previously fed with normal prey were resistant to frozen prey and showed high mortality. After 10 days of rearing with the enriched diet, when changed to a diet of alternatively frozen prey and live prey, an adjustment period with lower growth is observed. These results show that when juvenile cuttlefish are fed on live prey enriched with PUFA, they accept frozen prey earlier in their life and their survival is enhanced. These observations have a potential importance in the culture of juvenile cephalopods.

INTRODUCTION

In cephalopods, the juvenile stage is a critical period with high mortality due mainly to the transition between the embryonic nutrition mode and consumption of live prey (Vecchione 1987). Boucher-Rodoni *et al.* (1987) showed that after digestion of the yolk during the first three days of their life, juvenile cuttlefish must receive an appropriate diet or they die. Many investigations have been carried out on the culture of mature cuttlefish using alternative or artificial diets. Richard (1971, 1975), Pascual (1978), and Boletzky (1989) were among the first researchers who succeeded in culturing the european cuttlefish *Sepia officinalis* in the laboratory. According to Lee (1994), growth rates of young cephalopods cultured at 23°C can reach 13.5% BW.d⁻¹ (body weight per day). Hanlon *et al.* (1991) obtained daily growth rates over 10 months of 3-4% BW.d⁻¹, with a weight increase from 500 to 1400 g, at 20-24°C. Domingues *et al.* (2001a) cultured *S. officinalis* at 24-30°C and obtained daily IGR of 11.8% BW.d⁻¹ for hatchlings and a mean IGR of 2.5-8.5% BW.d⁻¹ throughout the life cycle (Domingues *et al.* 2001b). Good growth rates and low mortality have been obtained when juvenile cuttlefish were fed with live mysid shrimp during the first 10 to 20 days of the life cycle (Domingues *et al.* 2001a, 2001b, Koueta *et al.* 2002). Nevertheless, costs associated with mysid

production and their low fecundity (Domingues *et al.* 1998, Domingues *et al.* 2000) are a bottleneck in the first stage culture of the cuttlefish. In addition, mysid abundance in the wild varies considerably throughout the year, with periods of very low abundance. Experiments using *Artemia* sp. as first food resulted in poor growth (DeRusha *et al.* 1989, Domingues *et al.* 2001a) and high mortality (Pascual 1978, Navarro & Villanueva 2000). This is probably due to the lack of some lipids required for growth and survival of the cuttlefish (Navarro & Villanueva 2000). Furthermore, lower growth was obtained when cuttlefish were fed fish compared to feeding on grass shrimp (Domingues *et al.* 2003, 2004). Juvenile cuttlefish are predatory animals, their diet is essentially live prey. During post hatching development, they hunt exclusively small crustaceans, essentially mysids, *Gammarus* and young shrimps (Schröder 1966, Boletzky & Hanlon 1983, Boletzky 1989, DeRusha *et al.* 1989, Hanlon 1990, Pincson du Sel & Daguzan 1992, Hanlon & Messenger 1996). Many investigations on juvenile cuttlefish culture have demonstrated that several factors could influence the survival and growth of young animals, but the determinant factor to assure better health and thus better survival and growth is nutrition (Forsythe & Van Heukelem 1987, Clarke *et al.* 1989, Hanley *et al.* 1998, Koueta & Boucaud-Camou 1999, 2001, Koueta *et al.* 2002). Many attempts for rearing juvenile cuttlefish with frozen marine fish or frozen young shrimps have been

made, however the young animals fed on these diets accepted them only after 2 months (De Rusha *et al.* 1989, Lee *et al.* 1991, Hanley *et al.* 1998). Koueta & Boucaud-Camou (1999) indicated that only juvenile cuttlefish older than two weeks could be reared with frozen mysids. Castro (1991) did not succeed in culturing one week old cuttlefish with dead prey. Indeed when juvenile cuttlefish accept frozen prey, they are more fragile, and have a lower growth rate (Choe 1966, Boletzky & Hanlon 1983, Boletzky 1989, DeRusha *et al.* 1989, Koueta & Boucaud-Camou 1999). Domingues *et al.* (2002) showed that frozen shrimp is as good as live shrimp for culturing *S. officinalis*, even though total protein content of the diet were probably affected (leaching) by the freezing procedure. In fact it is still difficult in juvenile cuttlefish culture to use alternative or artificial diets during the first month of their life without the risk of great damage. To solve this problem, it is necessary to formulate alternative or artificial diets that are well accepted by juvenile cuttlefish in order to reduce their delay of live prey predation, and subsequently to investigate the total suppression of live prey during the entire juvenile phase.

Cephalopods have a high protein metabolism for their growth and energy production. Lee (1994) has suggested that proteins are the principal source of energy whereas fatty acids are mainly used for membrane constitution and functions, and for cholesterol and steroid hormone elaboration. The importance of polyunsaturated fatty acids (PUFA) on normal development of marine fish larvae and oysters has been shown by many investigators (Ostrowski & Divakaran 1990, Watanabe 1993, Ozkizilcik & Chu 1994, Barclay & Zeller 1996). Recent investigations on juvenile cephalopods have shown the impact of fatty acids on survival and growth. Navarro & Villanueva (2000, 2003) demonstrated that juvenile cephalopods need an important quantity of PUFA and especially DHA (22:6n-3) during their exponential phase of growth. In fact mortality and growth are correlated with the ratio of DHA/EPA (20:5n-3) in their nutrition. Koueta *et al.* (2002) demonstrated the importance of PUFA and especially DHA (22:6n-3) and EPA (20:5n-3) for the increasing of growth and survival of juvenile cuttlefish in culture. Domingues *et al.* (2004) showed that an optimal DHA/EPA ratio could be relevant for correct development of cuttlefish, and that variation in the concentrations of AA (20:4n-6) and EPA, essentially the ratio EPA/AA could have a profound effect on eicosanoid production.

In this study our objective was to test the effect of a natural diet enriched in PUFA (DHA and EPA) on the survival of juvenile cuttlefish receiving a frozen diet, and the impact on the acceptance of frozen prey during this critical period of their nutrition

MATERIAL AND METHODS

Experimental animals: Sexually mature females captured by trawl off the Normandy coast and maintained in a large tank laid their eggs in the laboratory. The eggs were placed on floating sieves distributed in a tank connected to the semi-closed system as previously described by Koueta & Boucaud-Camou (1999). Hatchlings were placed in small tanks of 707 cm² in groups of ten animals and fed *ad libitum* on live young shrimps.

Experiment: A total of 128 juvenile cuttlefish of maximum 2 days old, treated as described previously, were divided into 2 groups of 64 animals, housed and fed separately with the same quantity of food for each treatment and reared in the same conditions. Animals of group A (control) were fed on young shrimps and the remaining 64 of group B were fed on young shrimps enriched with fish oil. After respectively 5, 10, 15 and 20 days of rearing, frozen shrimp were used in each group to feed 16 animals. In this experiment, we had 64 observations per group and 16 observations per treatment in a group.

Fish oil: The omega-3 fish oil was provided by Dielen Laboratoires and contained respectively 18% EPA, 12% DHA of total fatty acids, 1mg/g of vitamin A, α -tocopherol (anti oxidizing agent) as indicated on the certificate of analysis.

Prey enrichment: Fresh shrimp surimi was totally soaked for 24 h at 4°C in Dielen Laboratoires fish oil. The enriched surimi was then distributed to young *Crangon crangon* at the rate of 5 g of surimi for 10 g of live prey as indicated in Koueta *et al.* (2002)

Compressed air was introduced into enrichment tanks from a number of narrow pipes opening on bubblers. These tanks were cleaned daily to avoid pollution related to over feeding or the quality of the diets.

Feeding methodology: Prey was offered once each day at 10 a.m. The daily ration (maximum ration) for juvenile cuttlefish as observed by Koueta & Boucaud-Camou (2001) was 40% of animal body weight. The daily ration was adjusted according to animal weight after 5 days during 30 days of rearing

Growth and food conversion rate analysis: The amount of food ingested by the individuals in each container was measured by weighing daily the food remaining in the individual tank. Weight and length measurements were made as described by Koueta & Boucaud-Camou (1999). Weight increase (mg) = Final weight-initial weight.

Statistical analysis: The statistical analysis was carried out using Statgraphics Plus software. Student's t-test was used to compare the mean values of each group at the same time between one treatment and the other animals. The results of different treatments were compared using ANOVA followed by multiple *a posteriori* comparisons employing the Tukey test (Sokal & Rohlf 1981). The multiple range test indicates which means are significantly different from the others with a significance level $p < 0.05$.

RESULTS

Survival in group A

After 30 days of rearing, survival rate was 75% for cuttlefish reared with young shrimps (group A). Mortality depended on the time of frozen prey feeding. When juvenile cuttlefish received frozen prey after 5 days, the survival rate was 56% at 10 days and 43% at 15 days of age. When juvenile cuttlefish were fed with frozen prey after 10 days, the survival rate was 75% at 15 and 50% at 20 days of age. When juvenile cuttlefish were fed with frozen prey at 20 days of age, at 25 and 30 days survival rate was 62%. They were more resistant. Mortality decreased when the change of the diet occurred later in the life of the animal (Table I, A, B).

Survival in group B

After 30 days of rearing the survival rate was 87.5% in the control group of cuttlefish reared with enriched prey (group B). The animals receiving frozen prey after 5 days had a survival rate of 68.75% after 10 days, 50% after 15 days and 31.25% reached 20 days of rearing. When frozen prey were given after 10 days, the survival rate was respectively 75% from day 15 to day 25 and 68.75% at day 30. When the frozen prey was given

after 20 days old, the survival rate was 81.25% at day 25 and 30. (Table I, A, B).

Survival is better in group B than in group A.

Growth in group A

During the rearing in this group, the cuttlefish that received the frozen diet after 5 or 10 days of age decreased in weight progressively and their behaviour was that of starved animals, they died respectively at 15 and 20 days of age. The growth rate of the juvenile cuttlefish that received the frozen diet after 20 days of age was significantly low compared to the control ($p < 0.5$) (Fig. 1 Top).

Growth in group B

During the rearing in group B only the cuttlefish which received the frozen prey after 5 days showed decreased weight dying at 20 days of age. The juvenile cuttlefish which received frozen prey after 10 or 20 days grew very well but the growth rate was significantly lower than in the control ($p < 0.5$) (Fig. 1 Bottom).

During the experiment, when the diet was changed, the juvenile cuttlefish needed a period of adaptation to eat and this induced a period of low growth rate.

Table I. – Top, Percentage of survival of juvenile cuttlefish during the rearing in group A (%). Bottom, Percentage of survival of juvenile cuttlefish during the rearing in group B (%).

	5 days	10 days	15 days	20 days	25 days	30 days
Live shrimps during 30 days (%)	87.50±0.09	81.25±0.10	75.00±0.11	75.00±0.11	75.00±0.11	75.00±0.11
Frozen shrimps after 5 days (%)	87.50±0.09	56.00±0.13	43.00±0.13	00	00	00
Frozen shrimps after 10 days (%)	81.25±0.10	81.25±0.10	75.00±0.11	50.00±0.13	00	00
Frozen shrimps after 20 days (%)	87.50±0.09	81.25±0.10	81.25±0.10	75.00±0.11	62.25±0.12	62.25±0.12
Total (%)	85.93±0.04	75.00±0.05	68.75±0.06	50.00±0.06	34.37±0.06	34.37±0.06

	5 days	10 days	15 days	20 days	25 days	30 days
Enriched shrimps during 30 days	100±0.00	93.75±0.06	93.75±0.06	87.50±0.09	87.50±0.09	87.50±0.09
Frozen shrimps after 5 days	93.75±0.06	68.75±0.12	50.00±0.13	31.25±0.12	00	00
Frozen shrimps after 10 days	93.75±0.06	87.50±0.09	75.00±0.11	75.00±0.11	75.00±0.11	68.75±0.12
Frozen shrimps after 20 days	100±0.00	100±0.00	93.75±0.06	93.75±0.06	81.25±0.10	81.25±0.10
Total (%)	96.88±0.02	87.50±0.03	75.00±0.05	71.88±0.05	60.94±0.06	59.38±0.06

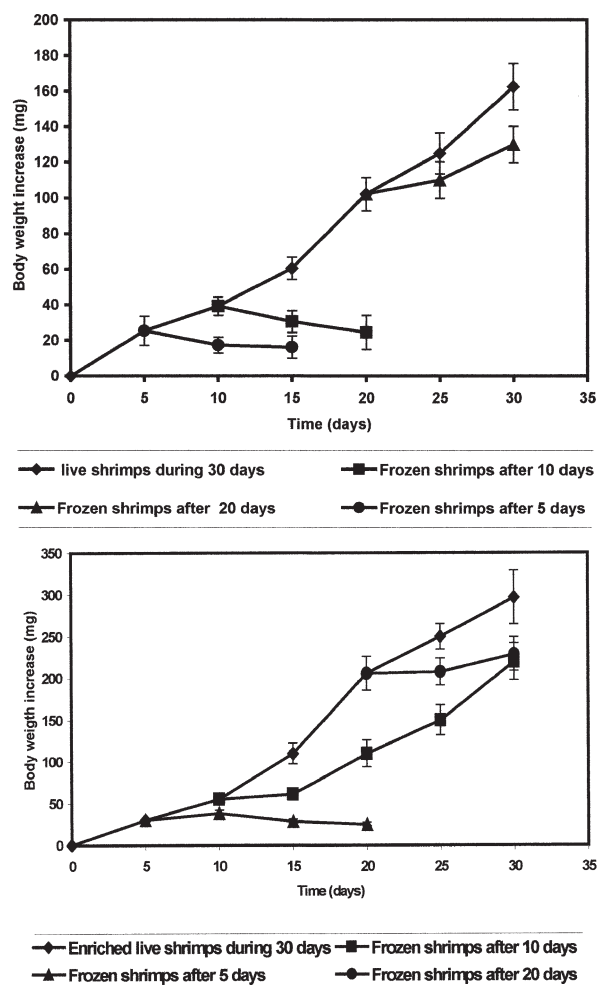


Fig. 1. – Top, Growth of juvenile cuttlefish (mg): effect of enriched natural diet on the delay of acceptance of frozen prey. Bottom, Growth of juvenile cuttlefish (mg): effect of enriched natural diet on the delay of acceptance of frozen prey.

DISCUSSION

Many investigations have shown that lipids are the main source of energy during the early development of molluscs (Gallager & Man 1986, Whyte *et al.* 1990, Delaunay 1992). The importance of polar lipids for marine molluscs has been previously described. DHA and EPA are essential for survival, growth and reproduction of *Crassostrea virginica* (Trider & Castel 1980) *C. gigas* (Waldock & Holland 1984), *Haliotis discus hannai* (Uki *et al.* 1986) and *Pecten maximus* (Delauney 1992). Koueta *et al.* (2002) showed the importance of PUFA and especially DHA and EPA in the nutrition of juvenile cuttlefish. Perrin (2004) demonstrated that shrimps enriched with fish oil had higher ratios of DHA/EPA and EPA/AA than the control, and that this enrichment induced growth increase. Domingues *et al.* (2004) have also shown

the correlation of the ratios DHA/EPA and EPA/AA and growth in cuttlefish. These results confirm our previous observations. The enrichment of the diet reduces mortality and stimulates growth in juvenile cuttlefish. The main observation in this investigation is that enrichment of the diet permits a reduction of the delay of acceptance of a frozen diet by juvenile cuttlefish and the mortality due to diet change. Despite the slower growth observed during the change of the diet from live prey to frozen prey, the enriched diet contributes to overcome this difficult period.

Lee (1994) has shown that the lipid content was low in cuttlefish (2% of body weight) and that the digestive gland served for stocking lipids. The diet enriched in PUFA for the juvenile cuttlefish could induce early maturation of the digestive system and modify the functioning of the digestive gland, hence the early acceptance of a frozen diet. The investigations of Yim (1978) and Boucaud-Camou *et al.* (1985) showed that the digestive gland of hatchlings is different from that of mature cuttlefish. The digestion is essentially intracellular during the first month. Perrin *et al.* (2004) showed that in juvenile cuttlefish fed with prey enriched with PUFA, acid phosphatase concentration was high. Acid phosphatase is one of the most important enzymes responsible for intracellular digestion in juvenile cuttlefish. Perrin *et al.* (2004) showed that the quality of food influences the development of the digestive gland of juvenile cuttlefish *S. officinalis*, as shown by Zambonino-Infante & Cahu (1999) in *Dicentrarchus labrax*.

This investigation showed that fatty acids are very important during the early life of juvenile cuttlefish by enhancing survival as observed by Cahu *et al.* (1998) and stimulating growth as observed in fish larvae by Sargent *et al.* (1997). PUFA are the precursors of prostaglandins and many hormones. They are also used in the constitution of cell membranes. Enrichment with PUFA may result in increased fluidity of cell membranes inducing better assimilation of nutrients. The fatty acids may permit the animal to react quickly to diet changes by activation or inhibition of digestive enzyme activities according to the physiological need of the animal.

When hatchlings of cuttlefish are fed with live prey enriched with PUFA, they accept very early in their life the frozen diet. This enriched diet contributes to reduce high mortality due to the change of the diet during rearing. The induction of early acceptance of frozen diet by juvenile cuttlefish using PUFA is essential for cuttlefish culture because it allows reduction of the difficult period of rearing when it is necessary to offer to the younger animals only live prey of an appropriate size. These investigations are also important for the future formulation of artificial diets for juvenile cuttlefish rearing.

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