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Food intake and growth in reared early juvenile cuttlefish *Sepia officinalis* L. (Mollusca Cephalopoda)

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Abstract

Experimental rearing of juvenile cuttlefish was carried out in a semi-closed system for 40 days at 19°C. Different quantities of live food were offered to isolated animals. The actual ingestion rate was enhanced by the amount of food offered, this tendency decreasing with age. Frozen food was ingested at the same rate, but was less effective than live food for growth. The quantity of food ingested during the first 20–30 days of life seems to affect further growth, especially weight increase. Aspartate transcarbamylase activity appeared to be a good indicator for the early phase of growth (first 20–30 days) which is likely due to hyperplasy, and moreover for predicting future growth.

Keywords: Cuttlefish; Culture; Food intake; Diet; Growth; ATCase

1. Introduction

Cephalopods are of great interest for biomedical research because their nervous system and sense organs involve large physiological capacities. Among cephalopods, the most popular models used for basic scientific investigations are *Octopus* and cuttlefish *Sepia officinalis*. In fact *Sepia officinalis* will become the 'white mouse' in many scientific laboratories and aquaria (Boletzky, 1989) because its organs and cells are increasingly used in electrophysiology, biomedical and environmental sciences. This fact is due to the easy conservation and transportation of eggs, the benthic habit of juveniles, good maintenance in the laboratory, and fast growth rate which permits adult animals to be obtained after only 1 year of life. Making use of such qualities, a number of investigations have been conducted during the last 20 years on electrophysiology

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(Fiedler and Schipp, 1991; Fiedler, 1992; Jakobs and Schipp, 1992), on the digestive gland and digestive mechanisms (Boucaud-Camou and Boucher-Rodoni, 1983; Koueta and Boucaud-Camou, 1986; Boucher-Rodoni et al., 1987; Castro et al., 1992), on behaviour (Messenger, 1977, 1981, 1991; Duval et al., 1984; Chichery and Chichery, 1992; Boal, 1996; Warnke, 1994; Adamo and Hanlon, 1996; Hanlon and Messenger, 1996; Dickel et al., 1997), on sexual maturation and its control by the optic gland (Koueta et al., 1992, 1993, 1995), on peptidic factors involved in the control of vitellogenesis and egg laying (Henry, 1993), and on post reproductive degeneration (Henry et al., 1994).

For most of these investigations, the cuttlefish used were trawled from the sea, then kept in the laboratory until experimentation. In this case, a regular and homogeneous supply is uncertain, and it is often difficult to maintain trawled animals for long experiments. To develop such investigations therefore, it appears necessary to obtain more animals reared in controlled conditions. Cultured animals can be easily used for in vivo experiments such as testing various growth, mitogenic, and vitellogenic factors. Knowledge of the background of the animals used appears crucial.

In the last 20 years, a number of cultures of cuttlefish in aquaria were successful using respectively live marine prey (Richard, 1975; Yim, 1978; Yim and Boucaud-Camou, 1980; Boucaud-Camou et al., 1985; Boletzky, 1989; Toll and Strain, 1988; Clarke et al., 1989), artificial diets (Castro, 1991; Castro et al., 1993), and alternative diets (DeRusha et al., 1989; Lee et al., 1991; Forsythe et al., 1994).

These investigations were mostly qualitative. Only few and scattered data are available concerning food ration and growth/ration relations. Indeed such data are needed to optimize a culture and also for field studies on recruitment. In this study we try to estimate the relation between food offered, food ingested, food conversion and growth in early juvenile (less than 2 months) cuttlefish, and we propose aspartate transcarbamylase activity (one of the enzymes of pyrimidine biosynthesis) as a biochemical growth index to estimate and predict early growth in cephalopods, as employed by Bergeron (1982) in fish, Bergeron and Alayse-Danet (1981) in scallops, and Erickson and Selivonchick (1987) in juvenile pacific oysters, *Crassostrea gigas*. This experimental study was carried out in a semi-closed system in which all environmental parameters were controlled.

2. Materials and methods.

2.1. Culture system (Fig. 1)

The semi-closed system limits the presence of particles and mud which cause turbidity of the water in the Bay of Seine. Furthermore, this system allows the renewal of 80% of seawater in the tanks per day, thus avoiding problems of evaporation, loss of water due to cleaning of the tanks, changes of salinity and pH, and also of nitrate, nitrite, and ammonium concentrations. In this system these different parameters are therefore the same as in open seawater and do not require any temporal adjustment when animal density is kept low.

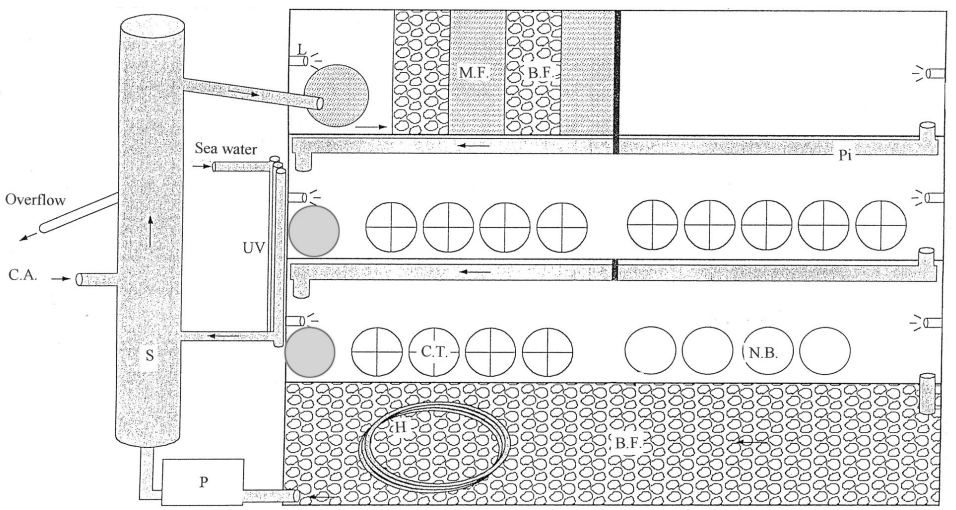

Fig. 1. Diagram of seawater and rearing systems. B, Lower conditioning tank; B.F., biological filter; C.A., Compressed air; C.T., Cylindrical tank; H, Heating; L, light; M.F., Mechanical filter; N.B., Tank for newly hatched individuals; P, Pump; Pi, Pipe; S, skimmer; S.W.; Sea water; U.V., u.v. lamps; →, Direction of seawater circulation in the rearing system.

2.2. Filtration system

Mechanical filters consist of foam and synthetic fibres packed in three tanks and placed in the superior tray against the running water. The water therefore seeps through the mechanical filters which alternate with three tanks of biological filters (oyster shells).

The tanks containing the filters are pierced with small holes allowing the water to flow slowly through the filters, ensuring proper filtration. The standardization of this filter system facilitates its change and rapid cleaning every day.

The base of the rearing system consists of a 1000-l reservoir tank containing a heating system. This tank is also filled with oyster shells as biological filters. Following this, the water is driven back into a skimmer by an automatic pump. The skimmer removes a maximum of dissolved organic material, then sends seawater back to the superior tray containing the filters previously described.

Before entering the system, the natural seawater which is used to renew the circuit runs through a system of three U.V. lamps with a flow rate of 55 l/h. (80% renewal per day).

2.3. Water circulation system

24 cylindrical tanks, 30 cm in diameter and 10 cm high, the bottom closed with nylon mesh (1 mm diameter), pierced with many horizontal 1 mm holes on the sides. The tanks are raised on 15-mm-high supports. These small tanks withstand the internal

horizontal and vertical circulation of seawater inside. Their small size, (707 cm^2) allows the alignment of many of these tanks in a basin of 2.38 m^2 containing 215 l of seawater.

2.4. Water oxygenation system

Compressed air is introduced into the system from a number of narrow pipes opening on bubblers surrounding each small tank. The aeration is permanent.

2.5. Light and temperature

The rearing device receives 12 h of light/24 h and the temperature of seawater is maintained at 19°C by the heating elements in the inferior conditioning tank.

2.6. Stoking and culture densities

The eggs used in this experiment were laid by two mature females trawled in the Bay of Seine during May and June, then kept in large tanks receiving natural seawater. The eggs were placed in floating sieves distributed in a tank connected to the semi-closed system previously described. The eggs were cleaned each day until hatching, which occurred after about 4 weeks. As hatching lasted several days, hatchlings were placed in the small tanks of 707 cm^2, in groups of ten animals according to their age and fed ad libitum on live mysids (*Mesopodopsis labberi* and *Schistomysis* sp).

A total of 80 cuttlefish were thus obtained. The cuttlefish were divided into four homogeneous groups of 1–10 days old animals, weighed, measured and randomly distributed in the small tanks of 707 cm^2. Each tank contained four animals well separated from one another by a thick partition, each animal thus having an area of 177 cm^2 and 2 l of seawater. Such isolation prevents aggression, competition, and cannibalism. The tanks were cleaned every day to eliminate dead prey and food remains.

2.7. Diets

The four groups (A, B, C, D) were reared on different diets. The first, second and third group contained 24 animals each, and the amount of food offered was respectively 21%, 30% and 35% of their body weight per day. These food quantities offered were adjusted every 5 days to the new mean weight of the group. Six animals of each group were harvested after respectively 10, 20, 30, 40 days of rearing for biological and biochemical analyses. The food, offered at 10 a.m, consisted alternatively of mysids, *Gammarus* sp, and young shrimps.

Group D contained eight animals, and food offered at 10 a.m. every day for 40 days consisted of frozen mysids, representing 30% of their body weight per day. The frozen mysids were offered only when the experiment was started. The walls of the containers were grey. The mean weight of the frozen mysids was 12 mg. Frozen mysids were chosen because they are more readily accepted by young cuttlefish than frozen schrimp or frozen *Gammarus*. The two containers used were placed near seawater inlet in the

second and first floors of the rearing system, thus keeping the frozen prey in continuous movement.

The cuttlefish were weighed and measured at the end of the experiment, killed by immersion in liquid nitrogen, and finally stored at −80°C until analysis.

2.8. Determination of food ingested (ration) and feeding rate

The amount of food ingested by the specimens in each container was measured by weighing each day the food remaining in the individual tanks.

The feeding rate (FR) was expressed as FR (% Body weight/day) = (FI/mean Wt × 100), where FI is the weight of food ingested by each animal during the time (t) of the experiment.

The conversion efficiency was calculated as : growth weight/weight of food ingested × 100.

2.9. Growth measurements

2.9.1. Length measurement.

A sliding calliper was used to measure dorsal mantle length with a precision of 0.1 mm.

2.9.2. Weight measurement.

A Sartorius balance was used to weigh all animals with a precision of 0.1 mg. Growth was expressed as 'Instantaneous relative Growth Rate' (IGR).

IGR (% Body weight/day) = $(\ln W_2 - \ln W_1) \times 100/t$, W_2 and W_1 are, respectively, final and initial weight (mg) of each animal, and t the duration of the experiment in days.

2.10. Preparation of samples for biochemical analyses

Samples of mantle (mostly muscular tissue) (80–100 mg) were used for measuring ATCase activity, and protein content. The samples were homogenized with 300 ml of chilled buffer containing tris 0.4 M/HCl 0.1 N, pH 9.5, 1mM EDTA (Ethylene diamino tetraacetic acid) and 1mM mercaptoethanol (Koueta et al., 1987), then centrifuged for 1 h, at 15 000 g, 4°C.

The supernatant was used for measuring ATCase activity and protein analysis.

2.11. Measurement of ATCase activity and protein content

The protocol used was based on that described by Bresnick and Mosse (1966) which was adapted by Bergeron and Alayse-Danet (1981) for *Pecten maximus*, and Mathieu (1985) *for Mytilus edulis*. The method was applied following the optimum conditions already determined by Koueta et al. (1987) for *Sepia officinalis*.

A volume of 150 μl of the previous supernatant was incubated (1 h at 35°C) with 200 μl of a ^{14}C aspartic acid (0.6 μCi/ml) solution and 100 μl of carbamyl-phosphate (9.5 mg/ml of Tris/HCl). The incubation was stopped with 100 μl of HCl 1N and the

solution centrifuged for 20 min (3000 *g*. 4°C). The ^{14}C carbamyl-aspartate produced was separated from the remaining ^{14}C aspartic acid by eluting 500 μl of the supernatant through an ion exchange column (Dowex 50) with 2.3 ml double-distilled water. Finally, the eluate was mixed with 4 ml of scintillation cocktail and the radioactivity (dpm) measured in a liquid scintillation counter (Intertechnic 5 L 32).

The ATCase activity was expressed as milli units (mU). One milli unit corresponds to one nmole of carbamyl aspartate formed per minute and per mg of protein.

A second sample (25–30 μl) of initial supernatant was used for quantification of protein related to ATCase activity. The assay followed the method of Lowry et al. (1951).

2.12. Statistical analysis

The results of different variables measured were compared between groups using Anova followed by multiple a posteriori comparisons employing the Tukey test (Sokal and Rohlf, 1981).

The correlation index was used to test the correlative change of ATCase index and IGR. The curve index was used to establish curves equation in order to compare mantle length growth, weight growth and change of feeding rate.

3. Results

3.1. Water quality

During the rearing experiment the pH was 8.24, the salinity was 33.5%°, nitrite (<0.01 mg/l) and ammonia (<0.5 mg/l) remained stable at all times without any chemical intervention. The renewal of seawater kept all parameters in the circuit close to those of natural seawater.

3.2. Survival

The rate of survival was 97%. Loss of some animals was observed only during the first 4 days of rearing, probably due to the stress of weighing and measuring procedures. Throughout the experiment, all of the animals stayed on the bottom of the tank and moved only to capture prey.

3.3. Effect of quantity of food offered on food ingested (Table 1)

The first observation is that food offered was in excess for the three groups, (respectively 21%, 30%, and 35% of body weight) as the amount of food ingested (ration) was always inferior to the food offered. Interestingly, the ration was higher in the group where large quantities of food was offered, this fact being more marked for the

Table 1
Change of feeding rate (ration) (% body/day) according to amount of food offered (F.O.) per day during the rearing (40 days)

Amount of food offered/day (% of body weight/day)	Time of rearing (days)	Ration (%)	Standard deviation	Number of animals used
21	10	15.78	1.41	5
21	20	11.89	1.27	5
21	30	10.95	1.07	6
21	40	9.26	1.23	6
30	10	27.23	2.96	6
30	20	19.15	3.45	6
30	30	13.62	1.51	6
30	40	10.84	0.80	6
35	10	30.84	4.20	6
35	20	21.94	3.59	6
35	30	14.91	1.22	6
35	40	12.57	1.18	6

youngest stages (30.84%) (Table 1) As shown, the ration ingested by the 35% group was twice that of the 21% group for the first 10 days of culture.

3.4. Growth/ration relationships (Figs. 2 and 3)

During the first 10 days of rearing, there was no significant difference in length or weight increase between the three groups. Afterwards, the 21% group showed lesser growth than the 30 and 35% groups. In these two groups, significant differences appeared in the weight curve after 20 days of rearing ($P < 0.003$) The conversion efficiencies for the three groups were respectively 46.71%, 38.15 and 37.85%.

3.5. Change of feeding rate with age (Fig. 4 and Table 1)

The ration decreased with age. The reduction was higher for the 35% and 30% groups. At the end of rearing, the animals were about 45–50 days old and had ingested a ration ranging from 9 to 13%.

3.6. Growth comparisons with cuttlefish fed with frozen prey (Table 2)

Group D, fed on frozen food, ingested a mean ration (11.76%) during 40 days, not significantly different from that of the control group (10.84%) fed on live prey. The conversion efficiency was nevertheless lower for frozen food (31.25%) than for live food (44.70%), thus the instantaneous growth rate remained low (4.62% against 7.33%). During the 40 days of culture the animals receiving live prey grew (in weight) twice as

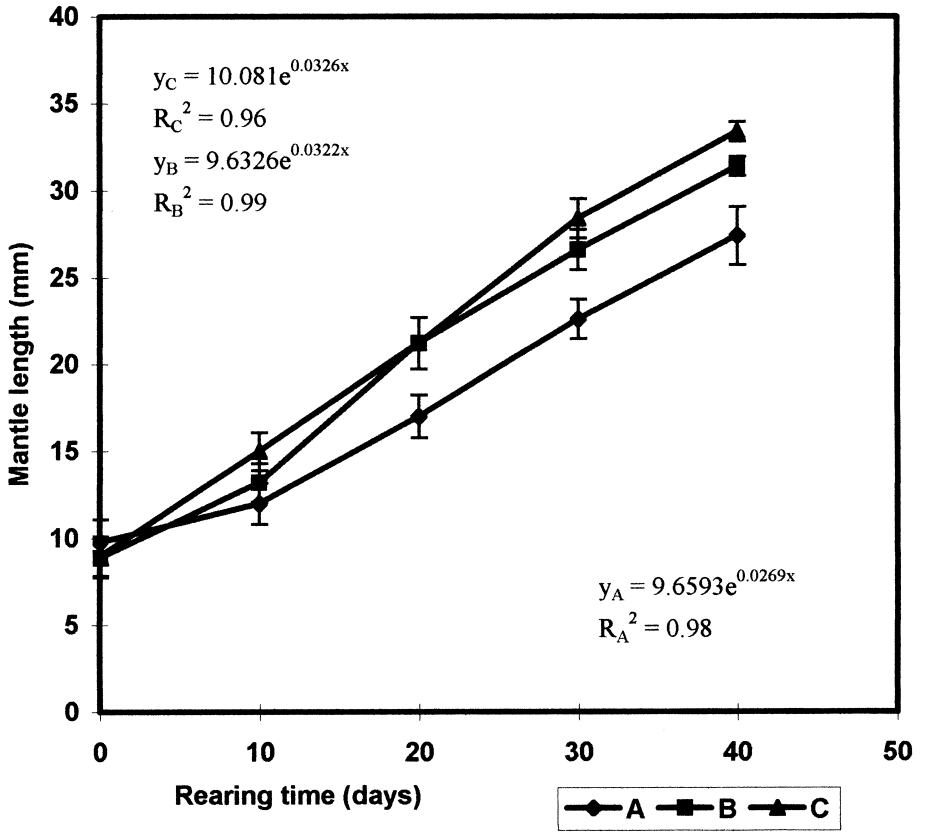

Fig. 2. Mantle length growth (mm) according to amount of food offered as percentage of body weight during 40 days of rearing. Tukey test ($P < 0.03$). A: 21%, B: 30%, C: 35%.

much as the group fed on frozen mysids. The difference in length, though significant, was less marked.

3.7. Measurement of ATCase activity in the mantle (Fig. 5)

For the three groups (A, B, C), the evolution of ATCase activity displayed the same trend : increase during the first 20 days of rearing, decrease thereafter. During the first month, the ATCase activity in group C was significantly higher than in groups B and A ($P < 0.003$).

3.8. ATCase activity, ration and instantaneous growth rate (Fig. 6)

ATCase activity was correlated to ration and to instantaneous growth rate only during the first 20 days. ($R_{10} = 0.98$, $R_{20} = 0.88$)

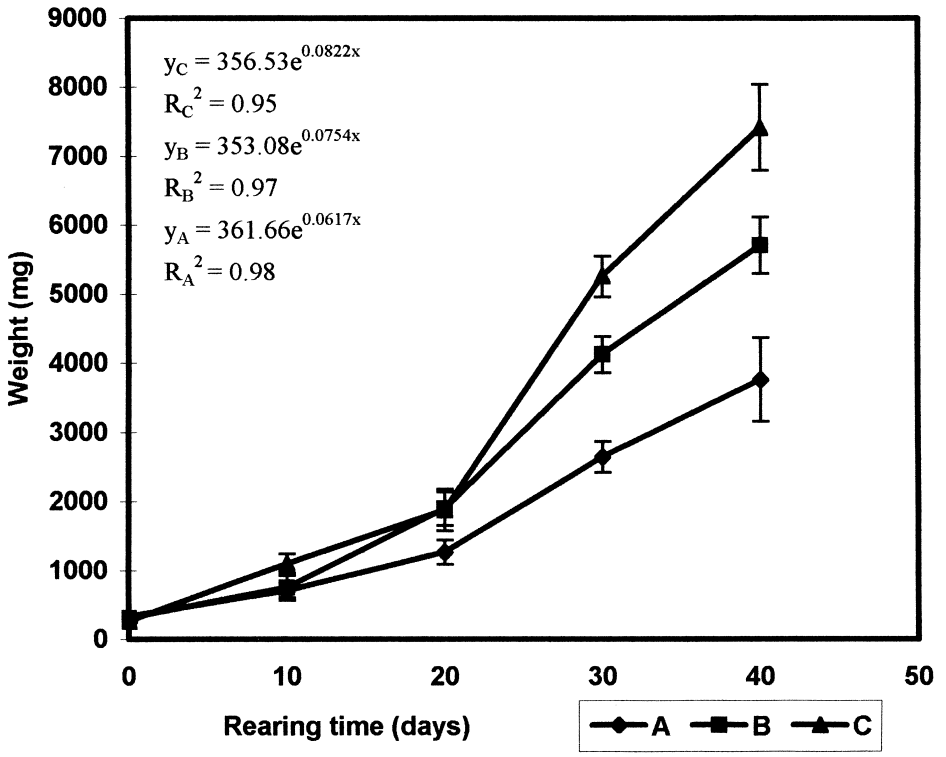

Fig. 3. Weight growth (mg) of young cuttlefish reared with different amount of food offered. (F.O.) during 40 days. Tukey test ($P < 0.03$). A: 21%, B: 30%, C: 35%.

4. Discussion

4.1. Rearing methodology: comparisons with previous data

For cuttlefish rearing, different authors, e.g. Schröder (1966), Richard (1971, 1975), Boletzky (1979) and Pascual (1978) have used an open seawater system during their experiments. In these cases however, the mortality rate was high, especially during the first 10 days after hatching, about 50% for Richard (1971, 1975) and 40% for Pascual (1978). DeRusha et al. (1989), Lee et al. (1991), Forsythe et al. (1994) and Warnke (1994) used a closed seawater system and survival exceeded 90%. Hanley et al. (1998), using a semi-closed basic system (9.7% seawater renewal per hour) reached 91% survival after 6 weeks of rearing. In this study we obtained 97% of hatchling survival in a semi-closed seawater system in which 80% of seawater is renewed each day. This result indicates that a semi-closed system is a good alternative when available (in marine laboratories). During the experiments, the salinity ($33.5^0/_{00}$), the pH (8.3), and the concentration of ammonia (< 0.5 mg/l), nitrite, and nitrate did not vary. This methodology requires less material and less control, and therefore is less expensive.

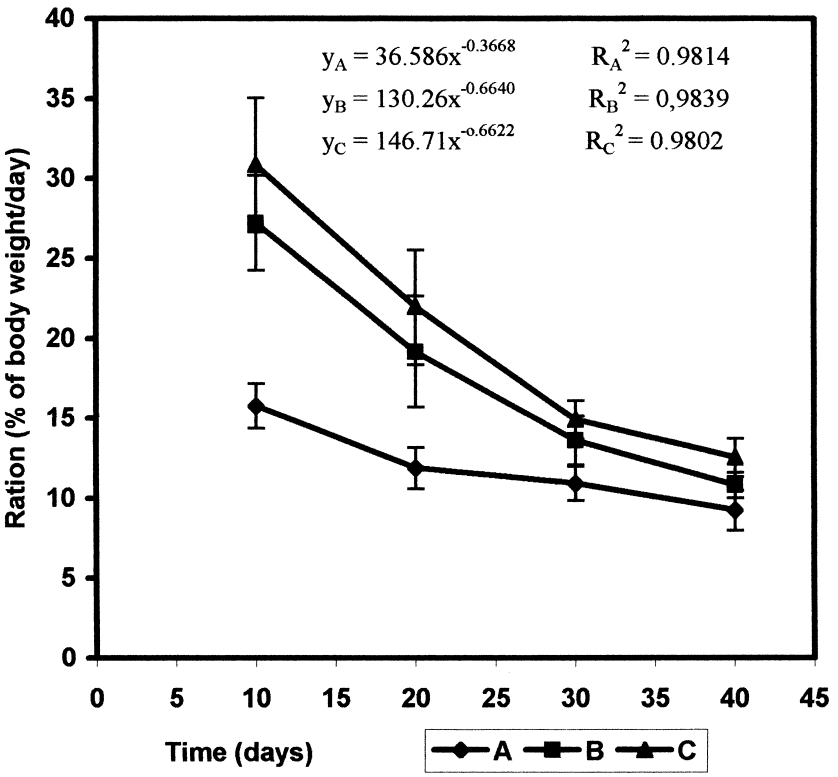

Fig. 4. Change of feeding rate (Ration) (% body weight/day) according to amount of food offered (F.O.) each day during 40 days of rearing. A: 21%, B: 30%, C: 35%.

Table 2
Growth comparisons between cuttlefish fed on live prey and cuttlefish fed on frozen prey (group B and D respectively)[a]

Diet	Frozen prey			Live prey		
variable	Mean	Sd	*N*	Mean	Sd	*N*
Iw (mg)	406.2	66.46	8	302.5	32.04	6
Iml (mm)	10.1	0.83	8	9.0	0.60	6
Fw (mg)	2452.8	286.01	8	5592.8	418.40	6
Fml (mm)	21.5	0.90	8	29.5	4.72	6
Mean weight (mg)	1426.5	102.32	8	2956.6	253.61	6
Weight gain (mg)	4.9	0.58	8	16.7	2.82	6
Food ingested (mg)	6577.7	487.74	8	12256.8	281.22	6
Growth rate (%)	4.6	0.19	8	7.3	0.43	6
Ration (%)	11.2	1.56	8	10.8	0.77	6

[a] Amount of food offered (30% of body weight/day). Rearing time: 40 days. Iw: initial weight. Iml: initial mantle length. Fw: final weight. Fml: final mantle length Sd: standard deviation. *N*: number of animals used.

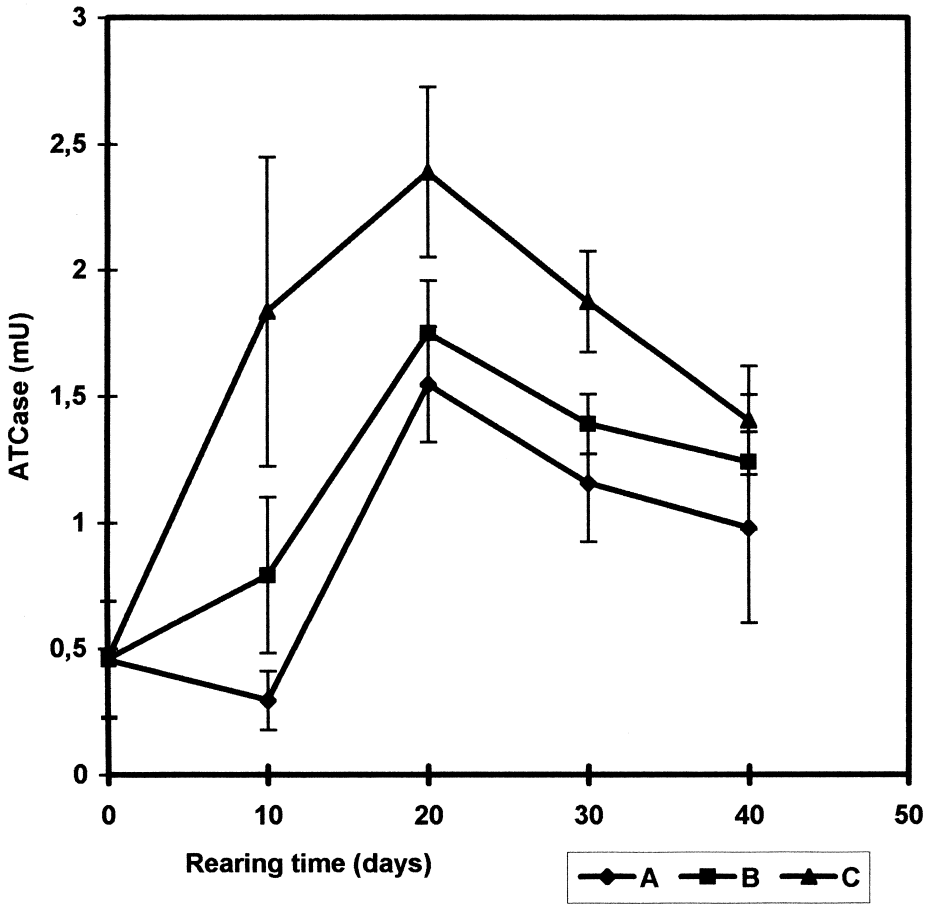

Fig. 5. Change of ATCase activity (mU) in young cuttlefish reared with different amount of food offered (F.O.) during 40 days. A: 21%, B: 30%, C: 35%.

Only the filtration system has to be cleaned every day to maintain the best water quality, a factor more important than inadequate feeding practices in avoiding mortality of these fragile animals in the Bay of Seine, where water quality is not good enough for the culture or the maintenance of this species.

In our previous work we noted more competition for food when juvenile animals were placed together, the weaker animals consequently dying. In this study the high rate of survival can be explained by animal isolation, eliminating intraspecific aggression and high competition for food. This methodology is therefore well suited to a study of ration and ration/growth relationship for isolated animals, as it eliminates the biotic competition factor, to study genetic differences for juvenile cuttlefish obtained from eggs of different area, and for the second generation. Furthermore, each animal had a large bottom surface area, and a water volume well suited for cuttlefish habits, and the experimental animals were not newly hatched specimens.

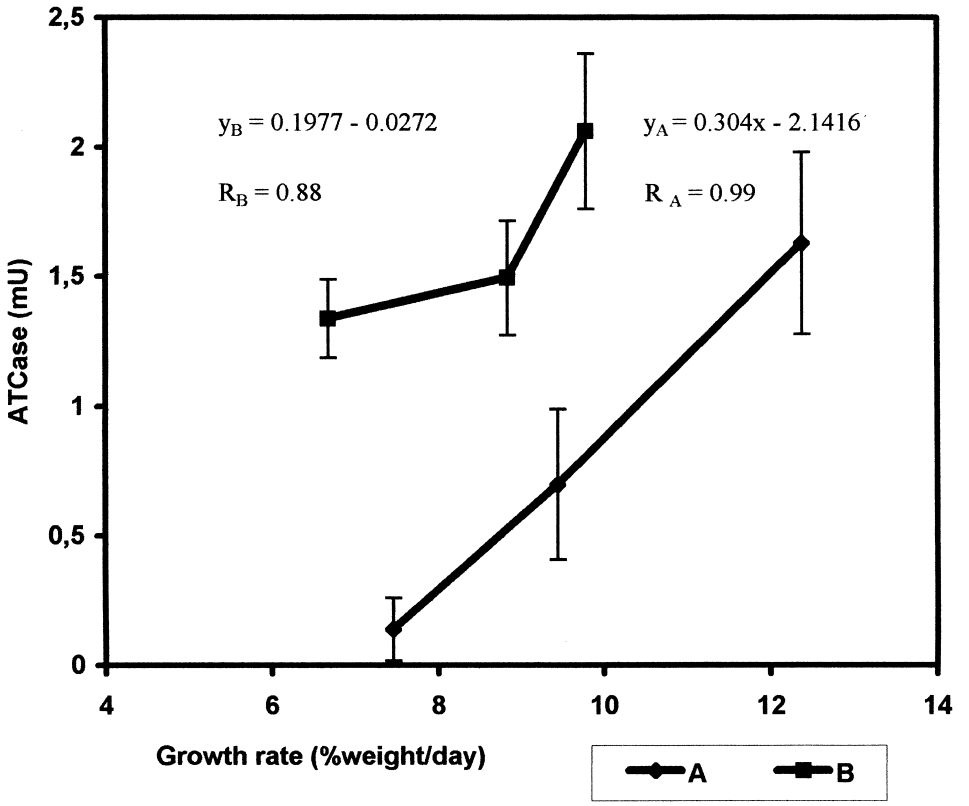

Fig. 6. Correlation between ATCase activity (mU) and growth rate (% weight/day). ($R_{20} = 0.88$, $R_{10} = 0.98$). A: 10 days of rearing; B: 20 days of rearing.

The only drawback of this methodology is that the number of animals in an experiment is limited, except in the case of very large installations, but it can be used as a pilot study in the development of larger scale experiments.

4.2. Amount of food offered and actual ration

Food offered (F.O.) was always in excess, the actual ration being always lower, and therefore all groups can be considered to be fed ad libitum. Actually, the quantity of F.O. affects the food ingested (F.I.), i. e. the ration, which is always higher for large quantities of F.O.. This is especially marked in young stages (10–20 days of rearing). This result suggests a stimulating effect of large quantities of food (in the absence of any competition). This stimulating effect is so marked that the F.I. for groups B and C is, during the first 20 days, in excess of the F.O. in group D (which is apparently overfed). This triggering effect seems only quantitative, as frozen prey is ingested at the same rate as live prey, and decreases with age. It could be related to the maturation of the short-term memory processes : early young cuttlefish ‘forget’ the prey once it is out of

their frontal field of vision (Dickel et al., 1997). A high density of prey could trigger capture by offering a higher probability of visual stimulations. During the first month of post-embryonic life, it is likely that physiological and ethological maturation occurs and explains the relative stabilisation in the amount of food ingested.

4.3. Growth comparisons (Table 3)

In this investigation the growth rate observed for group A after 40 days of rearing at 18–19°C (7.98 body weight/day)(bw/d) is higher than that of Pascual (1978) after 33 days of rearing at 21.6°C (5.43% bw/d) or after 46 days of rearing at 22.5°C (6.30% bw/d). DeRusha et al. (1989) found that the food weight needed by juvenile cuttlefish can reach 15–20% of animal body weight with a growth rate of 7.6% bw/d. at 22°C. In this study, early juvenile cuttlefish (group A) consume about 13% bw/d with a growth rate of 7.98% bw/d after 40 days. Comparison with available data (Table 3) confirms that mantle length and weight are higher in this investigation than in previous experiments, except for the work of DeRusha et al. (1989). However these growth performances still seem lower than those in the wild (unpublished results).

Not surprisingly the high food intake of both groups C and D (35 and 30%) correspond to high growth rates. However, this effect is delayed in time and is more marked in the last 20 days of rearing, when differences in F.I. between the three groups decrease.

In this study, we worked with excess food and the maintenance, optimum and maximum rations for each age group will have to be established. This will be necessary to optimize culture conditions. The growth–ration and growth–yield curves will allow the study of abiotic and biotic factors which affect growth of juvenile cuttlefish.

4.4. Growth on live or frozen diets

Lee et al. (1991) noted that small cuttlefish (20–30 g) require 3 days of conditioning with dead shrimp before readily grabbing non-living food. In our experiments early juvenile cuttlefish (350–400 mg) did not need conditioning delay to eat frozen mysids,

Table 3
Growth comparison between earlier data and present results: dorsal mantle length and weight at different temperatures. Early juvenile cuttlefish were fed ad libitum

Authors	Rearing temperature (°C)	10 days length (mm)	10 days weight (g)	Animal final age (days)	Animal final length (mm)	Animal final weight (g)
Richard, 1971	20	8.50	0.28	48	20.10	2.60
Richard, 1971	17	8.50	0.28	40	16.00	1.20
Yim, 1978	20	9.03	0.31	30	15.10	1.00
Pascual, 1978	20	10.10	0.45	40	17.00	1.20
Henry, 1993	18	9.10	0.31	42	17.00	1.50
DeRusha et al., 1989	22	–	0.45	40	–	4.20
Present work	19	8.80	0.27	40	21.20	2.10
Present work	19	8.80	0.28	50	33.40	7.40

because they had been fed for only 10 days with live prey. The delay noted by Lee et al. (1991) could be due to the fact that animals had received live food during all of their early development. Lee et al. (1991) showed that for juvenile cuttlefish of 420 g fed on live, frozen or pellet diet, growth is one third or less only for the group receiving pellet diet. DeRusha et al. (1989) working on 30–160 g juvenile cuttlefish found that the group receiving frozen diet grew 5–8% less during 1 month and 39% less during 2 months rearing. In this investigation we obtained 100% survival for animals fed on frozen diet, but the growth is 50% less than in group A. During this experiment we experienced difficulties in continually obtaining live mysids in order to maintain a control group fed only on live mysids. This lesser performance of growth using frozen food in juvenile cuttlefish will be further investigated. Indeed, these results show that frozen mysids, accepted by very young cuttlefish, could be an alternative diet to live food

The effect of a pellet diet on growth is more restrictive than a frozen diet. In mature animals, a frozen diet does not have any effect on growth (Lee et al., 1991). An alternative diet must be a food which can give growth performances comparable to live prey. DeRusha et al. (1989) and Lee et al. (1991) suggested that frozen prey could be used for cuttlefish rearing. For early juveniles, frozen mysids should be an alternative diet, but further research is needed on complementary food to be added to this diet in order to increase growth rate.

4.5. Use of ATCase as a biochemical growth index

In this investigation we observe a positive correlation between ATCcase activity, the ration and growth rate during the first 20 days of rearing, but there was no correlation afterwards.

ATCase activity appears therefore to be a good index for food intake during the first 20–30 days of life. ATCase is an enzyme involved in de novo synthesis of pyrimidine bases in fast growing tissues. In molluscs the activity of the enzymes was found to be associated with cell multiplication during gametogenesis (Bergeron and Alayse-Danet, 1981; Mathieu, 1985; Koueta et al., 1987). During post embryonic development of the digestive gland and digestive system of the cuttlefish, Yim and Boucaud-Camou (1980) observe an intense growth by hyperplasy. In the first month of life, intense cell multiplication does occur in young *Sepia* and the peak of ATCase activity probably is the marker of this growth by hyperplasy. Indeed, in this first phase the mantle length growth-rate is relatively low (mantle length gain : 66%; $y = 10.08e^{0.0326x}$). High food intake during this critical period is correlated with high ATCase activity and is followed by further high growth rate (weight gain 249%; IGR = 12.4; $y = 356.53e^{0.0822x}$) during the exponential phase of growth. It seems therefore that the hyperplasic growth governs the hypertrophic growth. In squid and *Idiopsepius pygmaeus,* growth occurs by combination of hyperplasia and hypertrophy (Moltschaniwskyj, 1994; Pelc and Moltschaniwskyj, 1997), but the new muscular fibres generated in larger squid are relatively fewer than in smaller squid, and the rate of new muscular block formation is more rapid in young *Idiopsepius pygmaeus*. This period of the first 20–30 days may be a crucial phase during which animals need a large amount of food to further develop a high growth rate. Thus ATCase appears to be a good marker to estimate early trophic

condition and moreover, to predict growth. Further studies are needed on unfed or underfed juveniles, if the validity of this index is confirmed, we will have a very good tool for recruitment studies in the field. However, a trophic index will be needed for the later stages : proteolytic activity, as used for fish larvae, could be a potential marker.

5. Conclusion

The semi-closed system we set up in the marine laboratory of Luc-sur-mer appears to be a good tool to develop experiments on feeding and growth in young cuttlefish since all environmental conditions are well controlled and stable. Such studies could be undertaken with five aims: 1) physiological research, 2) utilization of the relation found between the amount of food offered to the specimens and the current ration in cephalopod culture, and for the study of behavioural ecology of early stages of cephalopods 3) genetic studies on resistance, growth performance, and the fecundity of reared young cuttlefish obtained from eggs collected in different areas, and for the second generation. 4) all results obtained with isolated animals would be compared with those of animals reared in groups 5) development of a methodology suitable in the field for recruitment studies.

The preliminary results obtained in this work are very interesting in this respect. We show the triggering effect of large amounts of prey on food intake in early juveniles and its impact on both aspects of cellular growth, hyperplasy and hypertrophy. These results are in agreement with the opinion of Vecchione (1987) that the transition from yolk absorption to prey capture is most critical in the life history of cephalopods. Frozen mysids could be an alternative diet for young cuttlefish, and ATcase appears to be a good trophic index during the first stage of growth in cuttlefish.

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