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Recent advances in Mediterranean aquaculture finfish species diversification

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Culture of octopus (*Octopus vulgaris*, Cuvier): Present knowledge, problems and perspectives

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SUMMARY – This article reviews the results obtained over a four year period of research (1995-1999) at the Spanish Institute of Oceanography in Vigo, on the reproduction, rearing of paralarvae and on-growing of octopus. Optimum conditions for transport, stabling, feeding and induction to spawning with temperatures of 13 to 20°C are analysed, with salinity levels of 32 to 35‰. The biometric characteristics of the strings and eggs, fecundity, embryonic development, hatching and the rearing of 1.5 million of paralarvae are also noted, with a description of the form of feeding, growth and survival of the newly born paralarvae, using different types of prey: reared zooplankton (*Artemia salina*), fish eggs, wild zooplankton (copepods, cirripedia, mysidacea and euphausiacea), and zoeae of commercial crustaceans (crab, swimming crab and prawn) obtained from ovated females. In terms of the results obtained, there is discussion of the best conditions required for on-growing juveniles, regarding feed, daily ingestion, density of stabling and prior separation according to size and sex, attempting to optimize growth rates and feed conversion. Finally, some of the first experiences on on-growing at industrial level in Galicia in floating cages are described.

Key words: *Octopus vulgaris*, culture, reproduction, paralarvae, on-growing.

RESUME – "Culture du poulpe (*Octopus vulgaris*, Cuvier) : Connaissances actuelles, problèmes et perspectives". Cet article fait le compte rendu des résultats obtenus pendant quatre années de recherche (1995-1999) développée dans l'Institut d'Océanographie de Vigo, en relation avec la reproduction, la culture de paralarves et le grossissement du poulpe. On y analyse les meilleures conditions de transport, de stabulation, d'alimentation et d'induction à la ponte avec des températures qui varient entre 13 et 20°C et des taux de salinité d'entre 32 et 35‰. Les caractéristiques biométriques des grappes et des œufs sont aussi indiquées, de même que leur fécondité, leur développement embryonnaire et leur processus d'éclosion ainsi qu'une description de la forme d'alimentation, de croissance et de survie des paralarves nouvellement nées. Les résultats de la culture larvaire réalisée avec plus de 1,5 millions de paralarves avec différents types de proies sont également exposés et analysés : zooplancton de culture (*Artemia salina*), œufs de poissons, zooplancton naturel capturé dans la Ría de Vigo (copépodes, cirripèdes, mysidacés et euphausiacés) et zoés de crustacés commerciaux (crabe, étrille et crevette) obtenus à partir de femelles fécondées. Les conditions optimales nécessaires pour le grossissement des jeunes poulpes, relatives à l'alimentation, l'ingestion journalière, la densité de stabulation et la séparation préalable par taille et par sexe sont discutées sur la base des résultats obtenus, tout en essayant d'optimiser les taux de croissance et de conversion des aliments. Pour finir on présente une description de certaines des premières expériences de culture au niveau industriel développées en Galice dans des cages flottantes.

Mots-clés : *Octopus vulgaris*, culture, reproduction, paralarves, grossissement.

Introduction

Rearing of cephalopods is still in process of development (Boletzky and Hanlon, 1983). Common octopus (*Octopus vulgaris*) meets some of the requirements to be considered as a candidate for its industrial culture: easy adaptation to captivity conditions, high growth rates (Nixon, 1969), good acceptability of frozen food, high reproductive rate (Mangold, 1983) and high market price. However, only a few experiments of paralarvae culture of this species have been developed so far (Itami *et al.*, 1963; Villanueva, 1994), and they were performed on experimental scale.

The increasing demand of octopus in Asiatic and Mediterranean countries made the Spanish Institute of Oceanography in Vigo and the University of Santiago de Compostela to carry out a research during the period 1995-1999 to evaluate the viability of its culture. The main aims were: (i) to know the growth rate during the on-growing phase; (ii) to obtain paralarvae from the brood stock; and (iii) finally to rear these paralarvae. In this paper, a review of the results obtained at a pilot scale on

these fields are analyzed. Finally, the main problems of the culture and the use of this species for future commercial purposes are discussed as well as the most recent experiences of ongrowing in tanks (Iglesias *et al.*, 1997; Sánchez *et al.*, 1998; Otero *et al.*, 1999) and in floating cages (Rama-Villar *et al.*, 1997), which have encouraged the industry to start this phase of culture in Galician waters.

Material and methods

Reproduction

The reproducing stock comprised males and females in a 1:1 ratio, stabled in rectangular tanks with a capacity of 5 to 10 m³. Temperatures ranged from 13 to 20°C, with salinity from 32 to 35‰. The tanks were maintained with low light to obtain spawning as swiftly as possible. So that the females could deposit spawns, two types of dwelling were designed: PVC tubes (length = 40 cm, diameter = 20 cm), and PVC boxes (60 x 40 x 30 cm) open at one end, with a hinged lid to make it possible to observe inside. Once the spawns were obtained, the number of clusters, average length and the number of eggs per cluster were counted. Embryonic development was also controlled in certain spawns. Finally, fecundity and hatching rate of the eggs were estimated. In order to obtain highly viable spawns, a diet of frozen crustaceans (80%), fish (15%) and bivalve molluscs (5%) was given.

Culture of paralarvae

Over the four year period of the project, most of the research effort focussed on this objective as it is the bottleneck which, to date, hinders integral culture of this species.

The paralarvae are planktonic during the first weeks of life. At birth, they measure 3 mm in total length and require a diet of living organisms to cover the nutritional requirements at this stage. In the natural environment, in the course of this stage they feed of crustacean zoeae. Later, in their new benthonic habitat, food is more varied.

In the four year research period, diverse experiences with culture of 1.5 million paralarvae born in captivity were conducted, using different live prey: zooplankton from the natural environment (copepods, crustacean zoeae and mysidacea), reared zooplankton (*Artemia salina*), ichthyoplankton and, finally, crustacean zoeae obtained in laboratory from crab reproducing stock (*Carcinus maenas*), swimming crab (*Necora puber*) and shrimp (*Palaemon serratus*).

In order to conduct paralarvae culture experiences at a scale for future industrial application, 2000 l tanks were used with initial seed densities of 10 paralarvae per litre, with 24 hours of light. At 4 day intervals, the bottom of the tank was cleaned and a fourth of the volume was renewed. Temperature ranged from 18 to 20°C. Levels of oxygen, nitrites and ammonium were measured on a daily basis. Microalgae (*Isochrysis galbana* and *Tetraselmis suecica*) were introduced into the culture tanks, adjusting the concentration to 150,000 cell/ml. Considering the large amount of live prey required daily, it was decided to use *Artemia* as the only diet item: nauplii (0.5 mm) for the first week, followed by 1 to 4 mm of metanauplii cultured at 20°C for 7 days with said microalgae. Paralarvae were seeded in the culture tanks on the same day as hatching (day 0). Dry weight was recorded at regular intervals, sampling 30 paralarvae which, after being passed through distilled water, were dried at 55°C for 24 hours and weighed individually in a microbalance with a precision of 0.1 micrograms. Regular measurements were also taken of the lengths of the mantle and total lengths.

Growth of juveniles

In order to determine the growth rates for this species in terms of initial weight, sex and culture density, between 1995 and 1999 the following experiences were conducted.

Growth in terms of initial weight

Throughout the second half of 1995, 41 individuals were caught in the coastal area near the Oceanographic Centre. These individuals were divided into three groups with the following average

weights: 1340.0 g (Group 1), 580.0 g (Group 2) and 330.0 g (Group 3), with 10, 18 and 13 individuals respectively. Rectangular culture tanks were used, with 5 and 10 m³, with 1 m water height and in open circuit. Water temperature ranged from 13 to 19.5°C throughout this fattening period (August 1995 to June 1996), with salinity between 32 and 35‰, maintaining water flow at 1.2 m³/h. Food was supplied once a day, comprising: 80% crustaceans (*Polybius henslowi*, *Macropipus corrugatus*, *Carcinus maenas*), 15% fish (*Micromesistius poutassou*) and 5% of frozen molluscs (*Mytilus* sp.). Once a month, samplings were taken of the weight of each individual.

Growth by sexes

The objective of this experience was to improve the fattening process, rearing the males and females separately, thus avoiding fertilization of the females and subsequent natural mortality following egg hatching. Fifty seven octopi were separated according to sex, and transferred into two rectangular 8 m³ tanks, in similar conditions as those of the previous experience, where they were maintained for five months (December 1997 to April 1998). Average initial weights for males and females were 637.0 g and 657.9 g respectively. Monthly samples were taken to record weight. Separation according to sex was conducted by observing and touching the extremity of the third right arm. Diet comprised frozen decapod crustaceans. Daily ration was calculated as a percentage of the biomass in the tank. Growth curves for both sexes were subjected to a t-test to compare slopes. Light intensity was high in order to delay maturation of the females and to achieve a greater somatic growth. A photoperiod of 12 L:12 D was maintained.

Growth at different stocking densities

This experience was conducted to determine the effect of the initial load density on growth rate. To achieve this, fifty five individuals were separated into two batches, and transferred into two 1600 litre culture tanks, equipped with a water flow of de 0.02 m³/kg/h. Temperature range during the study period (November 1998 to March 1999) was from 13 to 16°C, with a salinity level of 32 to 35‰. The first batch (Group 1) comprised 37 octopi, with an average weight of 872.9 g, with an initial culture density of 20 kg/m³, whereas the second batch (Group 2) comprised 18 octopi with an average weight of 883.1 g, and initial culture density of 10 kg/m³. Cylindrical plastic parts were fitted onto the tank walls (diameter = 25 cm) in sufficient number so as to accommodate all the individuals. Fattening period was four months, during which samples were taken at two weekly intervals to record weight. The level of dissolved oxygen was also measured at regular intervals. Food, comprising crab (*Carcinus maenas*) and blue whiting (*Micromesistius poutassou*) in a ratio of 80:20, was supplied once daily, weighing the non-ingested remains. Daily ration was adjusted to 7% of the total biomass in the tank. The conversion index ($CI = FI/WG$, FI being the feed intake, WG the weight gain) and the specific growth rates [$G = \frac{\ln W_f - \ln W_i}{(t_f - t_i)} * 100$, where W_i is the initial weight, W_f being the final weight and t the time] were calculated. Finally, a comparison of the results on growth was made by a t-test to compare slopes. As in the previous experience, a photoperiod of 12 L:12 D was maintained, with a high light intensity.

Results and discussion

Catching and transporting individuals

Individuals were caught with no difficulty by contacting directly with the artisanal fisheries in various ports in the Ría of Vigo. Transport was in two ways: when the densities of the individuals were low (<10 kg/m³) and transfer time was no longer than 45 minutes, the octopi were introduced into plastic boxes with salty water and no aeration. For higher densities (of up to 50 kg/m³), they were stabled in large volume plastic tanks with oxygen supply. In this case, the octopi were introduced individually into plastic mesh bags, thus preventing the individuals from attacking each other.

Once at the laboratory, these were separated according to sex, observing the differentiated termination of the third right arm, which is shorter in males, and with the rounded extremity. A further external difference is the presence of two or three large suckers on the 2nd and 3rd pair of right and left arms, very evident particularly in adult males.

Reproduction

Reproductive behaviour is shown by the copulatory activity of the males, which insert the hectocotylus into the internal cavity of the females. When the latter are ready to deposit the spawn, they hide in dens, placing the clusters on the walls and roofs of the tubes or boxes. Spawning was noted in 100% of the stabled females.

It is advisable to separate the females from the reproducing tanks once they have deposited the egg clusters since they are far more calm and, in this way, it is possible to conduct a case by case follow up of each spawning. Throughout this period between spawning and egg hatching, the females stopped feeding, losing between 30 and 65% of their weight (Fig. 1). During this period, they did not leave the den, and cleaned and oxygenated the clusters, protecting the spawn.

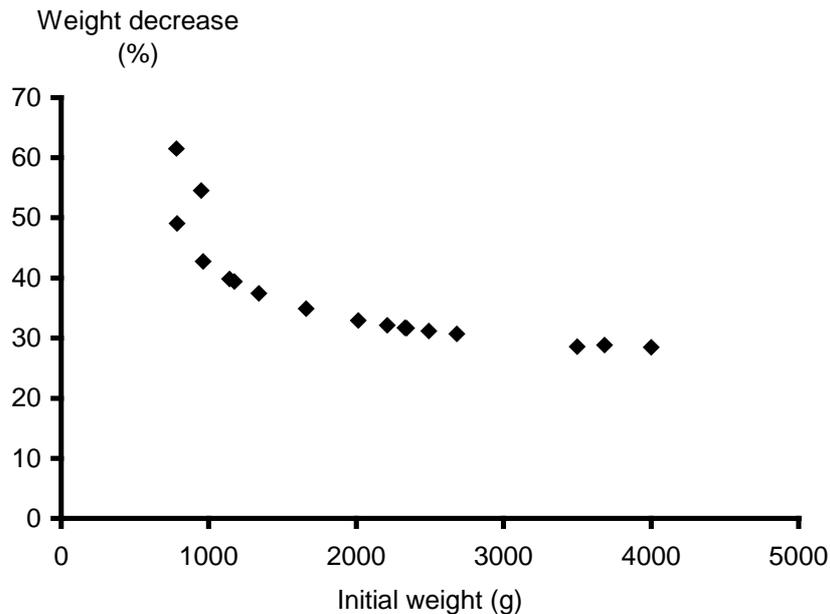


Fig. 1. Weight decrease of octopus females from the starting of the spawning period until the hatching of paralarvae.

According to Mangold (1983), the female is able to spawn from one hundred thousand to five hundred thousand eggs. The maximum number of eggs obtained in our reproduction experiences was 605,000 (Iglesias *et al.*, 1997). The general characteristics of octopus reproduction noted in our work, and the biometric parameters of the clusters, eggs and paralarvae are shown in Table 1.

Table 1. Characteristics and biometric data on octopus reproduction in culture conditions

Total number of females	20
Total number of spawns	20: 12 (in tubes), 8 (in boxes)
Weight of the females	2.7-8.3 kg
Spawning period	February-November
Duration of embryonic development	80-135 days
Number of clusters/spawn	180-550
Length of clusters	7.5-10.8 cm
Number of eggs/cluster	700-1100
Egg length at start of embryonic dev.	2.51 ± 0.05 mm
Egg length at hatching	2.90 ± 0.18 mm
Total paralarvae length (0 days)	2.95 ± 0.19 mm
Mantle length	1.49 ± 0.22 mm
Dry weight of the paralarvae (0 days)	0.46 ± 0.03 µg

Culture of paralarvae

The best results were obtained by using nauplii *Artemia* as prey during the first week of life, followed by metanauplii, measuring 1 to 4 mm, cultured at 20°C for a week with microalgae (Iglesias *et al.*, 1996). Under these conditions, the paralarvae increased their dry weight threefold in a period of 30 days, from 0.46 to 1.40 milligrams, with a 10% survival rate (Fig. 2). At the end of the experience, mortality was 100%. Cultures conducted with other types of live diets had a 100% mortality rate after a few days (Table 2). Itami *et al.* (1963) and Villanueva (1994), using shrimp zoeae (*Palaemon serrifer*) and hermit crab (*Pagurus prideaux*) as prey respectively, attained survival rates of 5% and 0.8% after two months of life.

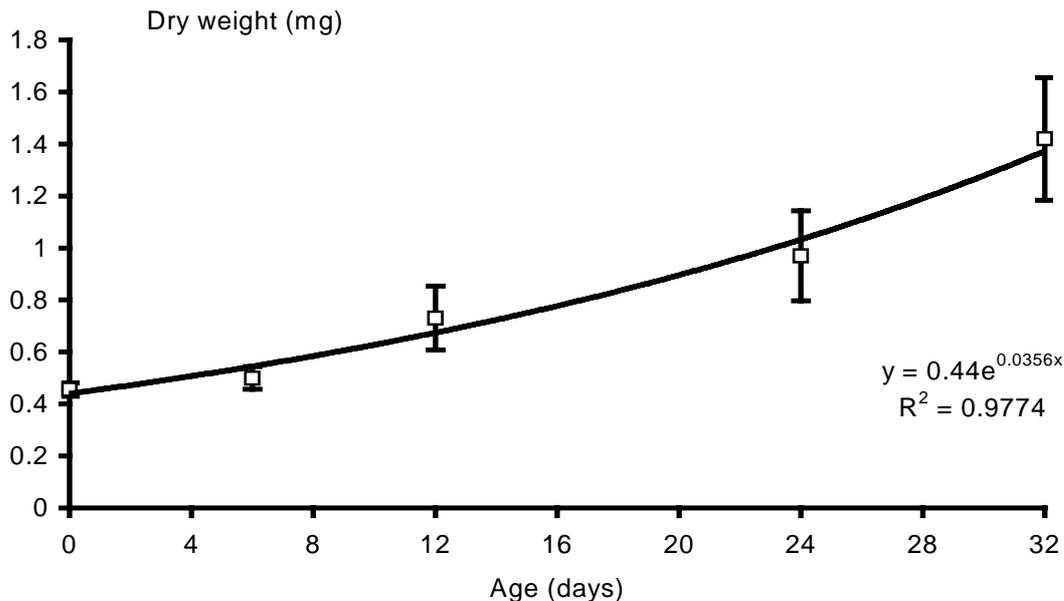


Fig. 2. Growth in weight of paralarvae of *Octopus vulgaris* fed with *Artemia* the first month of life.

Table 2. Rearing of paralarvae: description of the different trials

Trial no.	Tank vol. (l)	Initial number	Prey		Prey acceptability	Final age (days)	Survival (%)
			Type	Ind/ml			
1	100	500	Rotifers	2	–	4	0
2	100	500	Fish eggs	1	–	3	0
3	100	500	Micropellets	–	+	4	0
4	100	500	Copepods	0.2	+	4	0
5	100	500	<i>Artemia</i> ¹	0.5	++	8	0
6	100	500	Shrimp ²	0.5	+++	6	0
7	100	500	Crab ³	0.5	+++	8	0
8	2000	20000	1+2+3	0.5	+++	11	0
9	2000	20000	<i>Artemia</i> ¹	0.5	++	22	1
10	2000	20000	<i>Artemia</i> ⁴	0.5	+++	32	10

¹ *Artemia* = nauplii.

² Shrimp = *Palaemon serratus* zoeae.

³ Crab = *Carcinus maenas* and *Necora puber* zoeae.

⁴ *Artemia* = metanauplii.

In order to reduce these mortality rates and thus to close the culture cycle for this species, it will be necessary in future to focus research on finding prey with a more suitable nutritional profile and size, and to continue testing new enrichers with *Artemia* to meet the nutritional requirements of paralarvae more fully.

Growth of juveniles

Growth in terms of initial weight

The prime objective of the experience conducted with Group 1, which had the highest initial fattening weight, was to determine the maximum size obtainable in captivity. The result was an average weight of 12,300 g after 10 months fattening. Those with the lowest initial weight (Group 3) needed only 4 months to achieve a commercial weight of 2200 g. In the intermediate group (Group 2), a final weight of 5400 g was obtained after eight months. Figure 3 shows the growth in the three groups of cultured octopi. Daily ration ranged from 10% in the smallest, to 3% of the total stabled weight in the largest. Only a 3% mortality was recorded in the younger individuals of Group 3, coinciding with the first weeks of acclimatisation to captivity (Iglesias *et al.*, 1997).

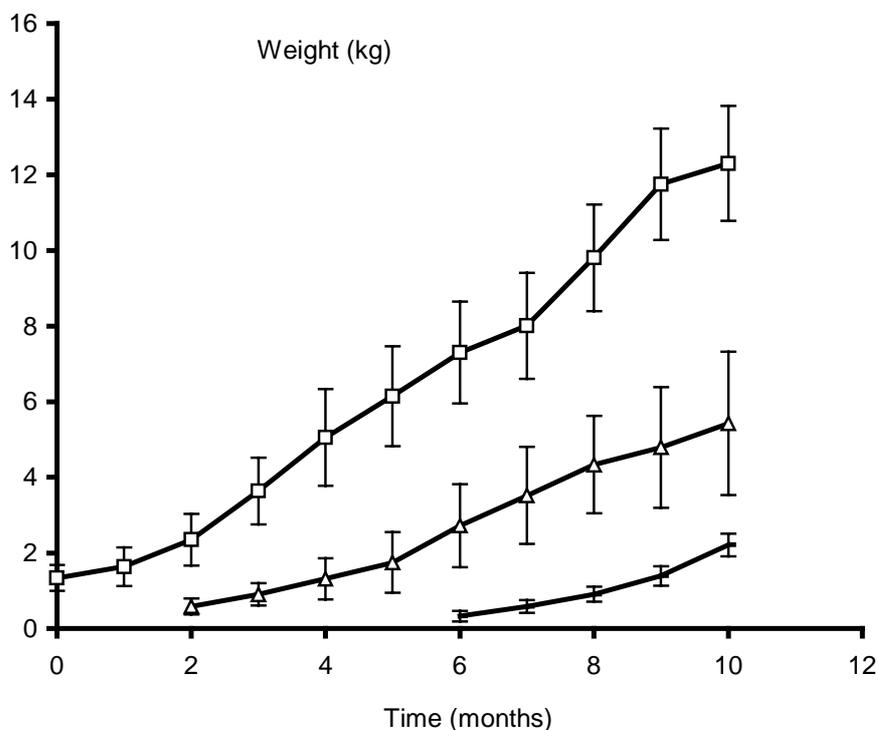


Fig. 3. Growth in three groups of octopi with different initial mean weights.

Growth by sexes

Growth rates in males were significantly higher ($p < 0.05$) than in females. After five months, average weight in males rose to 3624.0 g, whereas in females this rose to 2780.0 g (Fig. 4). Growth was adjusted to the following exponential equations:

$$\begin{array}{ll} \text{Males} & y = 544.11 e^{0.3327 x} \quad (r^2 = 0.75) \\ \text{Females} & y = 632.47 e^{0.2704 x} \quad (r^2 = 0.69) \end{array}$$

Daily ration was 7% at the start of the experience, which was adjusted monthly down to 3.5% at the end. Mortality rate during the first four months was 4% in males, and 6.7% in females. In the fifth month, this rate increased in both groups (13 and 10.7% respectively). Analysis of these results

reveals that separation according to sex improved culture yield as the females were not fertilised and continued to grow to commercial size. Also, considering the increase in mortality observed at the end of the process, it was concluded that to achieve best yield, it is inadvisable to prolong the fattening process beyond 3 kg for males and 2.5 kg for females (Sánchez *et al.*, 1998). Although it could not be demonstrated experimentally, the high luminosity used may have influenced the good growth rate recorded in the female tank. This fact (the influence of light intensity on maturation and spawning) has previously been noted by Mangold (1987).

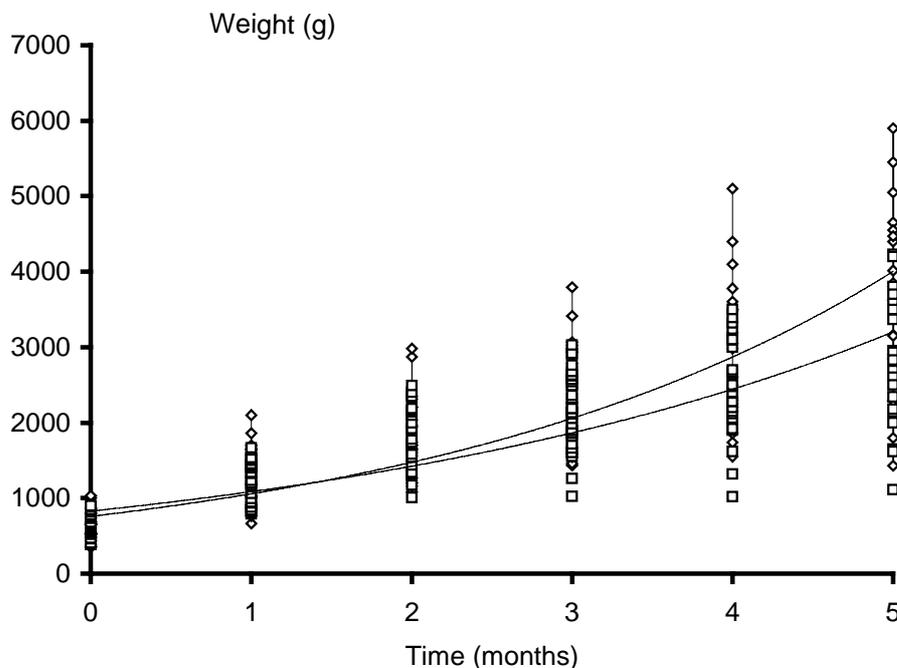


Fig. 4. Growth of males (diamonds) and females (squares) of *Octopus vulgaris* in separate tanks.

Growth at different stocking densities

In this experience, the final weights attained were 2205.0 and 2746.5 g for Groups 1 and 2 respectively. The slopes were significantly different ($p < 0.05$), growth in the tank with a lower initial density being higher (Fig. 5). The food conversion index was better in the Group 2. The final load densities were 45.5 and 29.2 kg/m³ in Groups 1 and 2. Mortality throughout the four months was lower in Group 2. Specific growth rates and conversion indices are shown in Table 3. Analysis of these results reveals that the fattening process improved by using an initial load density of 10 kg/m³ (Otero *et al.*, 1999).

Conclusions on the fattening process

In terms of our experiences, we may conclude that in order to obtain optimum results in fattening octopus, the following guidelines must be followed:

- (i) Stabling should be conducted with similar sizes.
- (ii) Initial density should not be greater than 10 kg/m³.
- (iii) Males and females should be separated.
- (iv) It is advisable to use artificial dens in equal number to the number of stabled octopi.

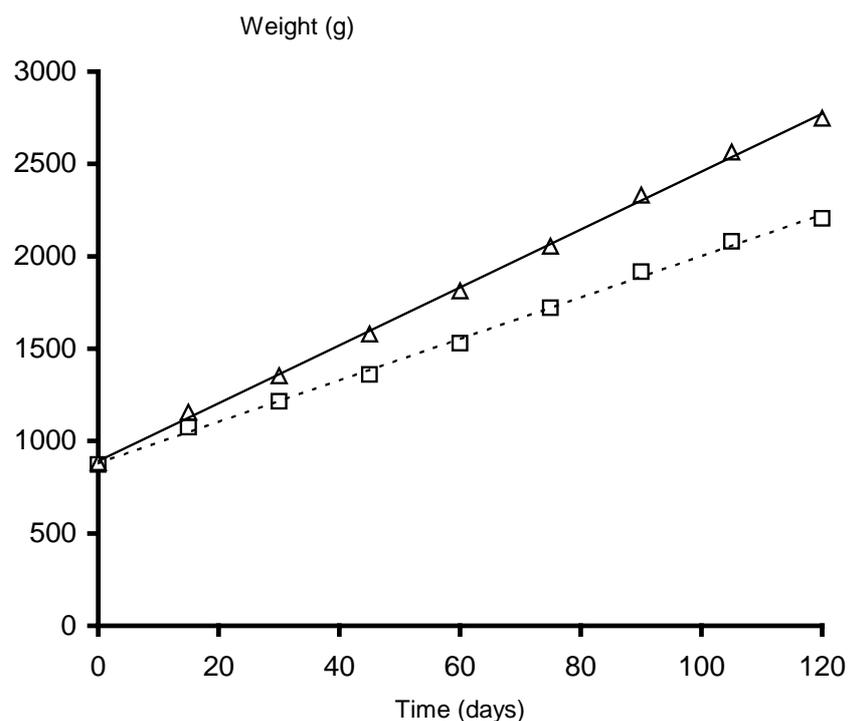


Fig. 5. Growth of two groups of octopi at different initial stocking densities: Group 1 (squares) at 20 kg/m³ and Group 2 (triangles) at 10 kg/m³.

Table 3. Specific growth rate (G), conversion index (CI), monthly mortality (MM) and stocking density (SD) of the two groups of octopi

	Months	G (%/d)	CI	MM (%)	SD (kg/m ³)
Group 1	0	–	–	–	20.2
	1	1.14	2.5	5.4	26.6
	2	0.79	2.1	2.9	32.5
	3	0.71	2.7	2.9	39.5
	4	0.48	3.7	0.0	45.5
	Total	0.77	2.7	10.8	–
Group 2	0	–	–	–	9.9
	1	1.47	1.8	5.6	14.4
	2	1.01	1.9	0.0	19.2
	3	0.79	2.3	0.0	24.8
	4	0.57	3.3	0.0	29.2
	Total	0.95	2.2	5.6	–

Under these conditions, no important problems of cannibalism or competition for food were observed. Taking into account that food is based on crustaceans and low commercial value fish, and with individuals weighing 750 g (minimum legal catch size in Galicia), it is possible to attain the commercial size of 2.5 to 3 kg in a period of three to four months, with a mortality rate not exceeding 10-15%, we may conclude that fattening this species is very interesting from the viewpoint of its industrial application.

Situation in the industry

Besides on the research conducted by the IEO on rearing octopus, Dr. Manuel Rey Méndez, of the

University of Santiago de Compostela and his team, carried out a fattening experience in floating cages in the Ría of Muros. Using frozen fish, such as sardine (*Sardina pilchardus*), horse mackerel (*Trachurus trachurus*), blue whiting (*Micromesistius poutassou*), sea bream (*Boops boops*), mackerel (*Scomber scombrus*) and molluscs (*Mytilus* sp.), similar fattening results were obtained with growth rates of 0.3 to 0.8 kg/month, and a mortality rate of 5.7% (Rama-Villar *et al.*, 1997).

Octopus is currently considered as a priority interest species for aquaculture in Spain, and a good deal of interest is being shown in various autonomous communities in terms of developing fattening projects, both in floating structures and in tanks. Specifically in Galicia there are several companies engaged in fattening octopus in floating cages.

The general exploitation layout in a company where these experiences are underway involves cylindrical or square shaped cages, with individual dens (on the walls or in the centre) with a capacity for 150 octopi. These cages may be individual units with an independent floatation system, or they may involve a common floating platform. The fattening process lasts 4 months, and three fattening cycles may be conducted during the year, attaining the commercial size (2.5-3 kg), so that a company with 25 cages may fatten about 11,000 octopi per year. Consequently, in this initial phase of development, the estimated total production in Galicia (NW Spain) is 50 MT, and the average price in the market is \$6 per kg.

In terms of reproduction and culture of paralarvae, more research work is called for to be able to close the culture cycle and not depend on catching juveniles in the natural environment.

Acknowledgements

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