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Effects of three culture densities on growth and survival of *Octopus vulgaris* (Cuvier, 1797)

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Abstract The purpoase of this research was to test the effects of three culture densities on the growth and survival of *Octopus vulgaris*. A total of 141 sub-adult octopuses (1,175.4 ± 194.9 g) were randomly distributed in nine tanks of 2,000 l each (3.6 m × 1.1 m, and 50 cm water depth). Three tanks were stocked with a low initial density of 4 kg/m^3, while three other tanks were stocked with an initial density of 8 kg/m^3, and the remaining three tanks were stocked at an initial density of 15 kg/m^3. Octopuses were all fed frozen squid (*Loligo gahi*) at 5% body weight per day (%BW/day). The experiment lasted for 70 days. Water temperature varied between 20 ± 2°C, and salinity varied between 36 ± 1 ppt. During the entire experiment, dissolved oxygen was always >75%, and ammonia was always lower than 0.1 mg/l. No differences in growth or growth rates (between 0.9 and 1.1%BW/day for the three densities) were found. Nevertheless, mortality was significantly lower for the low density compared to the other two densities tested. Maximum densities in the culture tanks (>25 kg/m^3) were attained in the higher culture densities after 56 days of the experiment.

Keywords Culture · Density · Growth · Mortality · *Octopus vulgaris*

Introduction

Octopus vulgaris is one of the best-known and most studied cephalopods. (Cagnetta and Sublimi 1999; Vaz-Pires et al. 2004). The species has a high food conversion rate (40–60%) (Wells 1978; Mangold 1983; Smale and Bouchan 1981), a fast growth rate, averaging 3% body weight per day (%BW/day) (Mangold and Boletzky 1973), and a high protein content, from 70 to 90% dry weight of its body composition (Lee 1994). Fecundity is very high, at 100,000 to 500,000 eggs per female (Iglesias et al. 1996, 1997).

The pioneers successfully cultured this species in Japan (Itami et al. 1963), obtaining benthic juveniles at 33 days. During the 1990s, Spanish scientists initiated research on

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O. vulgaris aquaculture, focusing both on paralarvae rearing and the on-growing of sub-adult individuals captured from the wild (Iglesias et al. 2007). The completion of the life cycle was achieved for the first time in northern Spain (Iglesias et al. 2002, 2004).

Octopus vulgaris is seen as a potential species for aquaculture diversification (García García et al. 2004). The on-growing of this species is currently being done in Galicia in northwest Spain (Iglesias et al. 1997; Rodriguez et al. 2003), with maximum annual productions in 1998 and 1999 lower than 33 *t* (Sánchez et al. 1998; Luaces-Canosa and Rey-Méndez 2001; García García et al. 2004; FAO 2002). The annual production decreased to 14.6 *t* in 2001 (García García et al. 2004) because of the dependence on captured juveniles from the wild and the absence of an artificial diet.

The major problems for the development of a solid and considerable commercial culture are the high mortality observed in the paralarvae stage (Iglesias et al. 2002, 2006, 2007), which prevents juvenile production, and the lack of artificial diets (Domingues et al. 2007, 2008).

The influence of diets during on-growing has been studied (Cagnetta and Sublimi 1999; Aguado and García García 2002; García García and Aguado 2002). Oxygen consumption when fasting and after eating was also determined (Cerezo and García García 2004a, b), as well as suitable oxygen concentrations at different temperatures (Cerezo et al. 2002; Cerezo and García García 2004a, 2004b).

Despite growing interest in the culture of this species, little information has been reported on the effects of culture densities. Lower growth at higher densities was reported for another cephalopod, *Sepia officinalis* (Domingues et al. 2003; Correia et al. 2005; Forsythe et al. 2002), while Otero et al. (1999) reported differences in food conversion and growth for juveniles of *O. vulgaris* stocked at 10 and 20 kg/m^3, but reaching final culture densities up to 45.5 kg/m^3, in a small experiment, at temperatures varying between 13 and 16°C. These authors suggest that initial density should not be higher than 10 kg/m^3.

The objective of this research was to determine the effects of culture density on growth and survival of sub-adults of *O. vulgaris*, cultured at temperatures averaging 20°C.

Materials and methods

Octopuses used in this study were captured using pots in the coastal waters of Huelva (south of Spain) in March 2008 and brought to the IFAPA "Centro Agua del Pino" research facilities (Cartaya, Spain). Animals were acclimated for 15 days prior to the experiment.

A total of 141 adult octopuses (1,175.4 ± 194.9 g) were randomly distributed in a flow-through system composed of nine tanks of 2 m^3 each (3.6 m × 1.1 m, and 50 cm of water depth). No significant differences ($P > 0.05$) were found in the weights of animals in the nine replicates. Each concrete tank had a solid mesh cover to prevent octopuses from escaping. Three tanks were stocked with a low initial density of 4 kg/m^3 (seven animals in each tank). Three other tanks were stocked with 8 kg/m^3 (14 animals in each tank), and the remaining three tanks were stocked at 15 kg/m^3 (26 animals in each tank). Octopuses were fed frozen squid (*Loligo gahi*) once a day at 14:00 h, at 5% body weight per day (%BW/day). The rations should be appropriate for on-growing of this species, based on previous studies by Aguado and García García (2002), García García and Aguado (2002), and Chapela et al. (2006). Food remains were collected from the tanks on the next morning and weighed previous to feeding. This allowed us to determine food ingestion. The food ration was adjusted after each weighing period. Water temperature was 20 ± 2°C, and salinity was

36 ± 1 ppt. Water flow was adjusted for the three densities in order to maintain water quality, with flows of 1,260, 1,080 and 720 l/h for high, medium and low density, respectively. These water flows maintained dissolved oxygen at >75%, pH between 7.9 and 8.1, and ammonia at <0.1 mg/l in the nine experimental tanks.

The experiment lasted for 70 days, because at this time matting behavior started to appear in every experimental tank. The octopuses were sampled after 3, 6, 8 and 10 weeks. All octopuses were weighed individually. For every sampling period, the weight data were used to calculate: (1) instantaneous growth rate (IGR) (% BW/day) $= ((\ln W_2 - \ln W_1)/t) \times 100$, where W_2 and W_1 are the final and initial weights of the octopuses, respectively, ln the natural logarithm, and t the number of days of the experimental period; (2) feeding rate (FR) (%BW/day) $= ((\mathrm{FI}/\text{Average } W(t)) \times 100$, where FI is the food ingested and Average W(t) is the average weight of the octopus during that period; (3) food conversion (FC) $= (W_2 - W_1)/\mathrm{FI}$, where $W_2 - W_1$ is the weight gained by the octopus during that period of time; (4) octopus density m^3 per tank = total weight of the octopuses (kg)/2. Mortality was calculated for each tank for each sampling period.

Statistical analysis was performed with ANOVA (significance at 0.05) to determine differences in weight, growth and feeding rates, and food conversions. When differences were found, a Tukey multiple comparison test was performed. The data were tested for normal distribution with the one-sample Kolmogorov–Smirnoff test and for homogeneity of variances with Levene's test (Zar 1999).

Results

Figure 1 shows the growth of octopuses cultured at the three culture densities. There were no significant differences ($P > 0.5$) in octopus weight between each tank, or each culture density, at each sampling. Final octopus weights were 2,310.4 ± 121.1, 2,419.2 ± 258.0 and 2,660.0 ± 331.6 g for octopuses cultured at the higher, medium and lower densities, respectively.

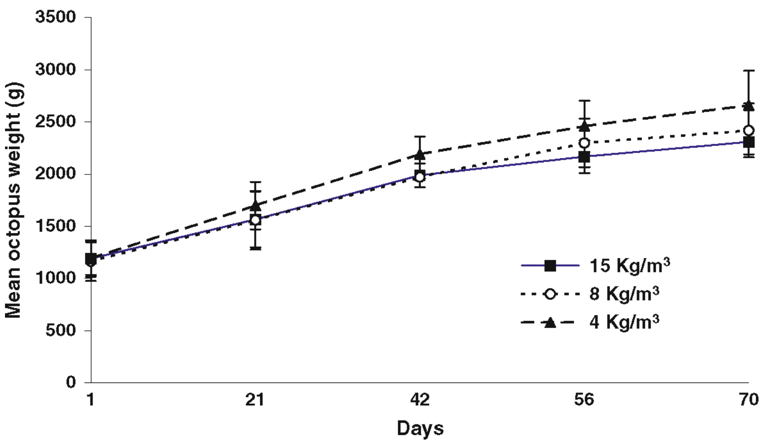

Fig. 1 Growth in weight (g) of sub-adult octopuses cultured at three culture densities for 70 days. *Bars* indicate standard deviations

Figure 2 shows octopus culture density (kg/m^3) throughout the experiment. The highest culture density was obtained in the initial high-density tanks and was 25.4 ± 3.0 kg/m^3 after 56 days of the experiment. From this period onwards, culture density decreased in the higher and medium density, and stabilized for the low density.

Figure 3 shows mortality for the experiment. Until day 56, there was no mortality at low or medium density, but at high density there was continuous low mortality throughout this period. Major mortality was observed during the last 2 weeks at high and medium density, even though water quality and temperature did not change, whereas only one mortality was observed within the three tanks at low density.

Growth rates (IGR) were only higher ($P < 0.05$) for low density during the first 21 days, and afterwards growth rates were similar at the three densities. Average growth rates for the entire experiment were 0.9 ± 0.2, 1.1 ± 0.2 and 1.2 ± 0.1%BW/day at high, medium and low densities, respectively, and were not significantly different ($P > 0.05$).

Feeding rates (Fig. 4) decreased consistently from almost 4 to 2%BW/day by the end of the experiment for all three densities. Feeding rates did not differ between culture densities on any of the sampling occasions. Overall feeding rates were the same ($P > 0.05$) for the three densities, being 3.1 ± 0.9, 3.0 ± 0.8 and 2.9 ± 1.1%BW/day for octopuses cultured at high, medium and low densities, respectively.

Food conversions (Fig. 5) differed significantly ($P < 0.05$) for the three densities tested and were 22.9 ± 3.0, 30.3 ± 5.9 and 37.7 ± 2.3% for octopuses cultured at high, medium and low densities, respectively.

Discussion

The on-growing of *O. vulgaris* was started in Galicia (northwest Spain) in the mid-1990s by small-scale companies with 750-g sub-adults captured from the wild (Rama-Villar et al. 1997; Sánchez et al. 1998; Iglesias et al. 2000; Luaces-Canosa and Rey-Méndez 2001; Iglesias et al. 2007).

Results for *O. vulgaris* on-growing have been reported in Spain (Iglesias et al. 1997, 1999, 2000), Portugal (Sendao et al. 1998) and Italy (Cagnetta and Sublimi 1999, 2000). Growth rates, close to 1% %BW/day, were similar to the ones reported for this species at

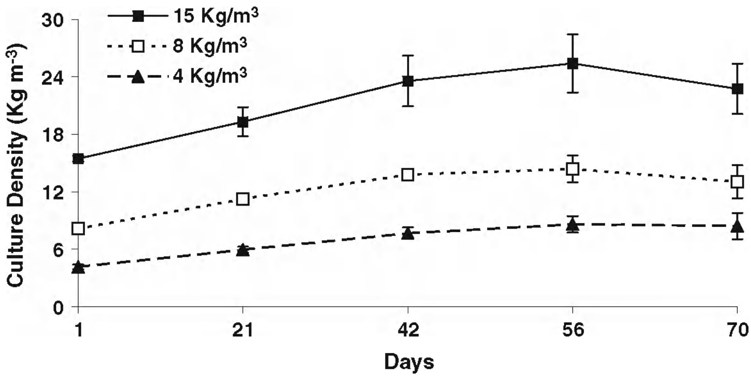

Fig. 2 Culture density per tank (kg m^{-3}) of sub-adult octopuses cultured at three culture densities for 70 days. *Bars* indicate standard deviations

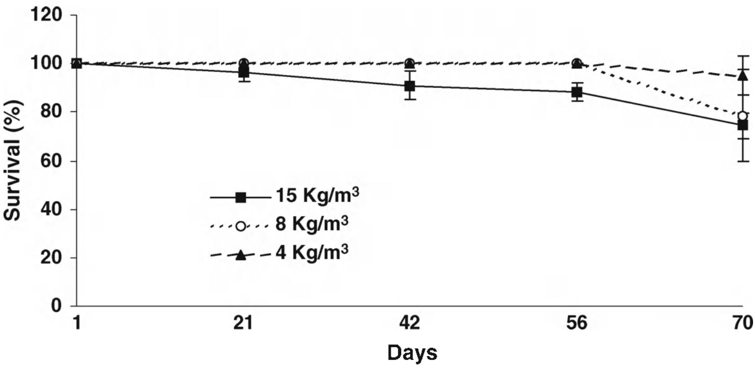

Fig. 3 Survival (%) of sub-adult octopuses cultured at three culture densities for 70 days

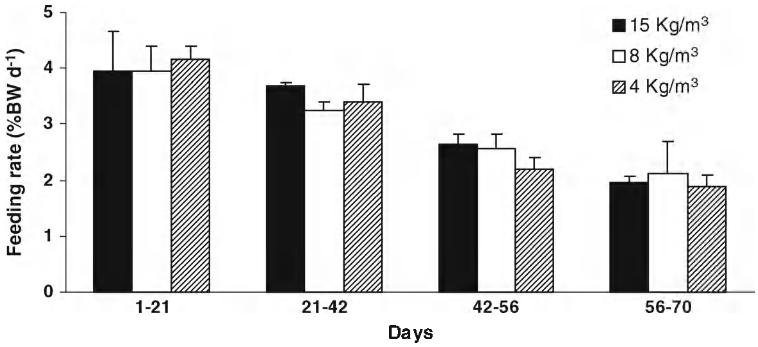

Fig. 4 Feeding rate (% body weight d^{-1}) of sub-adult octopuses cultured at three culture densities for 70 days. *Bars* indicate standard deviations

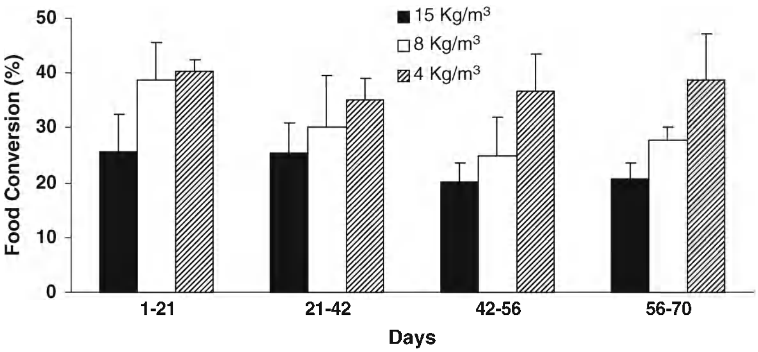

Fig. 5 Food conversions (%) of sub-adult octopuses cultured at three culture densities for 70 days. *Bars* indicate standard deviations

similar temperatures (Cagnetta and Sublimi 1999, 2000; García García and Aguado 2002) and were higher than those reported by Otero et al. (1999), Iglesias et al. (1999, 2000) and Chapela et al. (2006), who cultured this species at lower temperatures. Temperature greatly influences cephalopod growth (Domingues et al. 2005), food conversions (Domingues 1999) and ingestion (Domingues et al. 2006, 2007).

Although feeding rates decreased from 4 to 2% %BW/day for all densities throughout the experiment, this does not mean that octopuses ate progressively less food. In fact, food consumed was always similar throughout the experiment for all densities tested. The decrease in feeding rate is a reflection of the increase in weight of the animals; since this is a rate reported to correlate with the animal weight, when they grow larger, if they consume the same amount of food, this results in a decrease in feeding rates.

Typical food conversions between 15 and 43% are reported for this species (Mangold and Boletzky 1973; Vaz-Pires et al. 2004). Food conversions obtained for the three densities tested here fall within this range. Nevertheless, food conversions when cultured at the low density were higher and close to 40%, which indicates that the culture density was not affecting normal growth, and conditions were appropriate for the on-growing of those animals. Food conversions by octopuses cultured at high and medium density (22 and 30%, respectively) were significantly lower compared to those cultured at low density. Since feeding rates were similar for the three groups, this indicates that when density increases, octopuses use more of the energy consumed for metabolic purposes and less for biomass production. At higher densities, octopuses are probably more stressed and uncomfortable, move more, and are likely to use more energy in territorial confrontations.

Therefore, although not significantly, growth was also affected by density, as octopuses at decreasing culture densities were larger at the end of the experiment. The most probable explanation for the non-significance in weight at the end of the experiment among the three groups can be attributed to the large differences in animal weights in each tank, producing high standard deviations at the end of the experiment, preventing significant differences.

The fact that there were no significant differences in growth of *O. vulgaris* cultured at three different densities after 70 days was surprising for us. Even being aware of the weight dispersion typical of cephalopods, which increases standard deviations and therefore the difficulty in finding significant differences, we were expecting those differences; in cuttlefish (*Sepia officinalis*), bottom area and culture density do have a marked effect on growth (Forsythe et al. 2002; Correia et al. 2005). Perhaps the fact that *O. vulgaris* is more benthic and moves considerably less than *S. officinalis* makes octopuses more resistant to crowding than cuttlefish.

According to Cagnetta and Sublimi (1999), the use of a monodiet produces lower growth compared to feeding several prey of different groups, such as crustaceans and fish. According to these authors, crabs usually produced better growth than squid or fish. Nevertheless, we used squid since in previous years we had compared the growth of octopuses fed crabs and squid, and there were no significant differences between them (Domingues, personal communication). Also, using squid considerabley reduces the labor associated with tank cleaning. Furthermore, growth obtained here with a monodiet (squid) was not lower compared to that obtained when several types of prey were used (Otero et al. 1999; Chapela et al. 2006).

There was no cannibalism observed during the experiment, similarly to what was reported by Vaz-Pires et al. (2004) and Iglesias et al. (2000). Total biomass and consequent culture density in the tanks increased consistently until 56 days of the experiment in the three groups. Even the low mortality (only in the high density treatment) did not make total biomass or culture density decrease for this high density treatment. This is explained by the considerable increase of individual weight of the animals in the tanks. From day 56 until the end of the experiment, total biomass and culture density decreased in the high and medium density culture tanks. This was exclusively due to the mortality occurring during this period at these two densities. It appears that after 20–25 kg/m^3, there might have been some kind of compensatory mechanism that caused mortality of some individuals to prevent biomass from increasing linearly. This could result from hierarchical behavior, with larger animals not allowing or making it difficult for the smaller ones to feed at ease. This could be supported by the fact that mortality affected mainly smaller individuals. This same hierarchic behavior could also have contributed to the mortality in the medium density tanks, although culture density in these tanks was much lower ($\approx$12 kg/m^3). The existence of such "maximum culture density" for these tank designs and conditions could not be confirmed due to the start of mating behavior in all culture tanks, which made us finish the experiment at that time. In fact, reproductive behavior was first observed in some tanks after 60 days of the experiment, and in all tanks by day 70. When octopuses mature and start reproductive behavior, males grow larger because females experience stronger metabolic changes during sexual maturation, stopping somatic growth (O'Dor and Wells 1987; Iglesias et al. 2000; García García and Aguado 2002). This would compromise experimental results on growth and could also have contributed to the decrease in growth rates during this final part of the experiment, especially in the lower density where mortality was not a significant factor.

Otero et al. (1999) reported culture densities up to 45 kg/m^3; nevertheless, two factors could easily explain the much higher culture densities reported by this author: first, the fact that octopuses were cultured in the open sea, without water quality constraints or limitations, and the much larger size of the cages, which can allow a higher culture density, and second, the much lower temperature used by these authors (between 13 and 16°C), which considerably reduces metabolism and, consequently, aggressiveness. At 20°C, animals are much more active, and therefore culture densities have to be greatly reduced.

Previous information on on-growing of this species has been related mainly to cage culture in open sea, at average temperatures much lower than 20°C. Results reported here provide the first data for the culture of this species inland, at culture temperatures surpassing 20°C and at the minimum water-flows required to maintain water quality at acceptable levels.

Information collected during the present experiment indicates that appropriate culture densities for this species in concrete tanks should be significantly lower compared to cages in open sea, due to water quality limitations. Also, in Galicia, where culture is made at average temperatures much lower than 20°C, octopuses are collected at 750 g (minimum capture size), and on-growing is done until they are just over 3 kg in weight, when they are not still reproducing. We must take in consideration that at much higher temperatures (20°C), sexual maturity should be attained at much smalller sizes. During the present experiment, sexual maturity was attained at average weights below 2.5 kg. Since the minimum capture size in southern Spain is 1 kg, the margin of the on-growing period is greatly reduced. Therefore, further studies are necessary in order to determine if on-growing is economically appealing in such circumstances. One possible solution should be allowing the capture of smaller animals (e.g., 500 g) for on-growing purposes. One possible future experiment should consider this aspect and use smaller animals (below 750 g). The separation by sex prior to introducing animals in the tanks should also be tested. This procedure could possibly slow the maturation process and allow profitable on-growing until larger sizes.

In conclusion, we believe that for temperatures of 20 $\pm$ 2°C, the maximum sustainable density for the on-growing of *O. vulgaris* should not surpass 20 kg/m^3. An important aspect to take into consideration is the significantly higher ($P < 0.05$) mortality occurring in the higher culture density and the fact that it was not due to cannibalism. In our opinion, an appropriate culture density for this species must promote low mortality, this being of extreme importance when commercial-scale culture is the objective. Since water quality was similar for all densities, even after day 56 when mortality started to occur in the high and medium densities, we can only attribute this fact to the possible strong hierarchical behavior of this species, which might prevent smaller animals from feeding, or the fact that stress due to high densities (this is a solitary species by nature) could have contributed to a considerable decrease in the animals' defenses, which could cause mortality. This is also a crucial aspect strongly indicating that for higher temperatures, initial lower culture densities must be used due to the territorial and aggressive nature of this species.

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