

Effects of Different Prey and Rearing Densities on Growth and Survival of *Octopus Maya* Hatchlings

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Abstract

The present study aims to determining the isolated and combined effects of stocking and prey densities on growth and survival of *Octopus maya* hatchlings both at experimental level and in a pilot scale system (8 m²; 2700 L). Octopus survival was not related to prey density. Gained wet weight resulted in a significant interaction between initial stocking density and prey density indicating that octopus growth under low and high density was affected in a different manner depending on the density in which prey were offered. Prey density did not have a significant effect on growth and octopus fed with all three prey densities gained wet weight in a similar way. Results indicate the use of culture densities of 140 octopus m², and at least 0.27 g prey octopus⁻¹ d⁻¹ can be used to cultivate octopuses in small tanks. In tanks of 8m² a higher growth rate was obtained with both 25 and 50 octopus m² densities were used. Survival was not affected by stocking density between 25 to 75 octopus m².

Keywords: Cephalopods; Cannibalism; Culture densities; Growth rate; *Octopus maya*; Prey densities

Introduction

The Yucatan octopus, *Octopus maya* [1], is an endemic species from the Yucatan peninsula. Distribution ranges from Ciudad del Carmen, in the North of the Yucatan peninsula, to Isla Mujeres [2]. It has direct embryonic development with high hatchling survival [3], and easily adapts to laboratory conditions, accepting dead prey or prepared diets immediately after hatching [4,5]. *O. maya* has been cultured in the laboratory [6-9] up to four [7] or five [10] generations. The Yucatan octopus is characterized by fast growth rates (up to 8% body weight, [BW] d⁻¹), high feeding rates and food conversions that vary between 30% and 60% [4,10]. This species grows to 1 kg in 4 months at 25°C, attaining maximum weight (>3 Kg) at 9 months old [10].

The determination of optimum culture densities is a major aspect for the optimization of large scale culture of cephalopods [11,12]. There is information reported on density studies for several cephalopod species, but none for *O. maya*.

It is known that both juvenile and adult *Sepia officinalis* tolerate high stocking densities well [13,14], and juvenile *O. vulgaris* also tolerates high densities, with similar growth rates at lower and higher stocking densities, although a higher mortality was observed in groups maintained at high density [11]. Although density studies have been conducted for cephalopods, [12-15], the combined effects of culture and prey density have never been analyzed

The effects of prey density on cephalopod growth and behaviour has been poorly studied and understood, especially when using hatchlings. To our knowledge, a pioneering investigation by Borer [16] was partly devoted to study the functional response of adult octopus (*Octopus briareus*), while Koueta & Boucaud-Camou [17] reported data on the relationship between the amounts of food offered and ingested in *S. officinalis* juveniles. Nevertheless, prey density is a key factor in optimizing growth and survival. In fact, an interval of prey densities for which density-dependent prey mortality is probable, was reported by Marquez et al. [18] when studying the functional response curve of *S. officinalis* hatchlings preying on live mysids. Rearing density studies for the cuttlefish were also reported by Correia, Domingues, Sykes & Andrade [19]. The effects of prey density were reported for *O. vulgaris*

paralarvae [20] while growth and prey consumption by *S. officinalis* fed different densities of live mysid shrimp was reported by Sykes [14].

During the first 15 days after hatch, *O. maya* hatchlings pass through a transition process characterized by physiological and morphological changes associated with the maturity of the digestive gland, use of remaining yolk and changes in proportion of arms related with total length [21]. This period was also identified as a “no net growth” on squid [22]. For that reason the pilot scale culture of *O. maya* has been divided in two parts: pre-fattening and fattening, the first in hatchery/nursery facilities and the second in outside ponds [23]. During the pre-fattening period octopuses are maintained in 8 m² ponds for 60 days, where they are fed with crab paste and *Artemia* adults.

The present study was divided in three experiments in an attempt to

- i) Determine the isolated and combined effects of stocking and prey densities on growth and survival of *O. maya* hatchlings (Experiments I and II), and
- ii) To evaluate the effect of stocking densities on growth and survival of *O. maya* hatchlings maintained in a pilot scale system (Experiment III). All the animals used in the experiments were hatchlings of 2 days old.

Material and Methods

The study was carried out in the Experimental Cephalopod Production Unit (EPHAPU) at the UMDI-UNAM, Sisal, Yucatan, Mexico, following the procedures of Rosas and Moguel [5,21] for

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collecting and maintenance of egg-laying females, as well as rearing, and maintenance, of *Octopus maya* embryos. The octopuses were obtained from spawning of females copulated in controlled conditions [23].

Experiments I and II were carried out in tanks with seawater maintained at $26 \pm 2^\circ\text{C}$; pH between 8.0 and 8.2; dissolved $\text{O}_2 > 5.5$ mg/L; and a natural photoperiod of 10-14 h light-darkness (300 ± 50 lux cm^{-2}). Live shrimp (*Palaemonetes sp.*) was used as prey, which were collected in a daily bases in ponds around the research facilities (Sisal, Yucatan, Mexico). Individual shrimp wet weight varied between 0.1 and 1.3 g with a mean individual value of 0.60 ± 0.01 g ($n = 500$). Food was provided daily at 10:00 h and 14:00 h, and uneaten live shrimp and shrimp leftovers were removed and weighed every day prior to the first morning feed, in order to determine feeding rates. Thorough tank cleaning was performed on a daily basis.

Experiment I: Effect of initial stocking density and prey density on survival and growth

A two-way factorial design was used to test the effect of two octopus stocking densities (140 and 280 octopuses m^{-2} , corresponding to 50 and 100 octopus aquaria⁻¹, respectively) in combination with three prey densities (2, 4 and 6 prey octopus⁻¹ d^{-1} , corresponding to 0.09, 0.18 and 0.27 g prey octopus⁻¹ d^{-1} , and 71, 141 and 226% of initial body weight, respectively) on octopus survival, growth and feeding rate. Three replicate tanks (70.4 L; 76 cm x 47.5 cm x 19.5 cm) with re-circulating sea water were randomly allocated in each of the 6 treatment combinations. A total of 1350 hatchlings of *O. maya* (2 days old) were used in this experiment (Table 1). Each tank had empty shells of adults *Melongena corona bispinosa* (3 shells per octopus) to reduce stress by providing shelter for experimental animals to hide. A total of 7100 g of shrimp were used as live prey throughout the duration of the experiment (30 days).

All animals were individually weighed using an Ohaus® semi micro scale (± 0.001 g) at the beginning and at the end of the experiment. Individual mean wet weight gained was calculated by subtracting the mean final weight of the mean initial weight in each tank.

Specific growth rate (SGR, % day^{-1}) was calculated as:

$$\text{SGR, \% day}^{-1} = [(\text{Ln } W_f - \text{Ln } W_i)/T] \times 100$$

Where W_f is the final weight (g), W_i is the initial weight (g), and T is time in days.

Survival in each tank was obtained as the difference between the number of octopus at the start and end of the experiment. Ingestion rate was calculated as a percentage of food provided (IR_{ipt}) and as a percentage of final biomass production (IR_{ibp}) obtained in each tank:

$$\text{IR}_{\text{ipt}} = (\text{Ingested food, g tank}^{-1} \text{ d}^{-1} / \text{Food provided, g tank}^{-1} \text{ d}^{-1}) \times 100$$

Using ingestion rate and biomass production per tank the food

Experiment I		Prey density (g octopus ⁻¹ d ⁻¹)		
		0.09	0.18	0.27
Stocking density (number of hatchlings m^{-2})	140	0.136 \pm 0.012; n = 150	0.130 \pm 0.010; n = 150	0.124 \pm 0.018; n = 150
	280	0.134 \pm 0.051; n = 300	0.124 \pm 0.021; n = 300	0.115 \pm 0.015; n = 300
Experiment II		0.124 \pm 0.004; n = 10	0.125 \pm 0.010; n = 10	0.125 \pm 0.006; n = 9

Table 1: Initial wet weight of *Octopus maya* hatchlings (2 days old) that were randomly allocated to different stocking and prey density treatments in experiments I and II. Values are mean \pm standard deviation; n is the number of weighed individuals.

conversion index (FCI_i) was calculated as:

$$\text{FCI}_i, \% = \text{Total ingested food (g tank}^{-1} \text{ 30 d}^{-1}) / \text{Total octopus production (g tank}^{-1} \text{ 30 d}^{-1}) \times 100$$

The tank gross growth efficiency ($\text{GGE}_i, \%$) was defined as the fraction or the percentage of food intake by animals in the tank for 30 d [24] that was converted into body mass in the tank (ΔBW_i) during experimental time:

$$\text{GGE}_i = \Delta\text{BW}_i / \text{FI}_i$$

Survival, IR_{ipt} , FCI_i , GGE_i and SGR (% day^{-1}) were analysed by means of two-way ANOVAs with octopus stocking density and prey density as the two main (fixed) factors ($n = 3$ replicates). An arc-sin transformation of data was performed prior to statistical analysis [25]. Comparisons amongst means were carried out using the Student - Newman - Keuls procedure for balanced designs. Statistical differences were assumed when a $P < 0.05$ was obtained [26]. Growth rates were finally expressed as specific growth rate (SGR % bw d^{-1}) for 30 experimental days for comparative purposes. Statistical analysis was done using R software.

Experiment II: Effect of prey density on growth

A total of 30 hatchlings of *O. maya* (2 days old) were used in this experiment (Table 1). Animals were individually placed in 30 plastic 500 ml chambers (10 cm diameter) connected to re-circulating sea-water. Each chamber had one empty shell of the mollusk *M. corona bispinosa* in order to provide shelter and reduce stress. Similar prey densities as for experiment I were tested (0.09, 0.18 and 0.27 g prey octopus⁻¹ d^{-1}), using $n = 10$ individual replicates in each treatment.

All animals were weighed at the beginning and at the end of the experiment, and individual wet weight gained was calculated. Specific growth rate (SGR , % day^{-1}) was calculated as:

$$\text{SGR, \% day}^{-1} = [(\text{Ln } W_f - \text{Ln } W_i)/T] \times 100$$

where W_f is the final weight (g), W_i is the initial weight (g), and T is time in days.

Ingestion rates were calculated as percentage of food provided per animal (IR_{ipa}):

$$\text{IR}_{\text{ipa}} = (\text{Ingested food, g animal}^{-1} \text{ d}^{-1} / \text{Food provided, g animal}^{-1} \text{ d}^{-1}) \times 100$$

Using ingestion rate and biomass production per tank the food conversion index (FCI_i) was calculated as:

$$\text{FCI}_i, \% = \text{Total ingested food (g animal}^{-1} \text{ 30 d}^{-1}) / \text{Total octopus growth (g animal}^{-1} \text{ 30 d}^{-1}) \times 100$$

The individual gross growth efficiency ($\text{GGE}_i, \%$) is defined as the fraction or the percentage of individual food intake (FI_i ; $\text{g animal}^{-1} \text{ d}^{-1}$) that is converted into body mass and can be expressed as:

$$\text{GGE}_i = \Delta\text{BW}_i / \text{FI}_i$$

where ΔBW_i is individual growth ($\text{g animal}^{-1} \text{ d}^{-1}$).

One-way ANOVAs [25] were performed to determine differences in gained weight and food ingestion rates. Mean values were then compared using Tukey's HSD test for unequal samples. Individual growth rates were, here again, expressed as SGR (% bw d^{-1}) for 30 experimental days. Statistical differences were assumed when a $P < 0.05$ was obtained.

Experiment III: Effect of stocking densities on growth and survival of *O. maya* hatchlings maintained in a pilot scale system

A total of 4800 hatchlings of *O. maya* (2 days old) with similar initial weight as for experiment I (0.13 ± 0.01 g, $n=500$) were used in this experiment. Animals were randomly placed in ten 8 m² black tanks (2,400 L; $2 \times 4 \times 0.3$ m) under the following stocking densities: 3 tanks with 25, 50, and 75 octopuses m⁻² each, and one tank with a density of 150 octopuses m⁻². Each tank was connected to a re-circulatory sea-water system coupled to anthracite vertical filter and protein skimmer [23]. Each tank was provided with 3 *M. corona bispinosa* shell per animal. During the 30 days of the experiment, animals were fed *ad libitum* two times a day (09:00 and 18:00 h) with crab paste (95% lyophilized crab meat plus 5% gelatin without flavor; [5], at ration of 150% of octopus wet weight. Also, *Artemia* adults (25 g wet weight per tank d⁻¹) were provided during the first 15 days [21]. *Artemia* were collected in salty ponds from the Celestun coastal lagoon, Yucatán, México. Before feeding (08:00 h), tanks were cleaned and remaining food removed using a siphon. Seawater in the tanks was maintained at $26 \pm 2^\circ\text{C}$, 8 ± 0.5 pH, dissolved O₂ > 5.5 mg/L, nitrite < 0.05 mg/L and ammonia < 0.5 mg/L; A natural photoperiod of 10-14 h light-darkness, respectively was maintained during the experiment. At the end of the experiment, all animals were weighed and counted to calculate the final wet weight biomass and survival obtained in each experimental condition.

The relationship between biomass (g) of *O. maya* juveniles after 30 days of culture and initial density (number of octopuses m⁻²) was explored using additive modelling (GAM). This was done after data exploratory analysis revealed a non-linear relationship between response and explanatory variables. Biomass data was previously square root transformed to comply with the Gaussian family of distribution curves. Exploration analysis used point graphs to identify extreme data; histograms and percentile graphs to assess normality; and X-Y graphs to verify linear relationships. Since there were only 4 values for the covariate (25, 50, 75 and 150 octopuses m⁻²), the model with the maximum amount of smoothing used 3 degrees of freedom (d. f.), whereas, the model with the minimum amount of smoothing used d. f. = 2. The difference amongst these was assessed by means of AIC (Akaike Information Criteria = $-2 \log(\text{Likelihood}) + 2df$) and hypothesis testing using the *F* statistic. The model was validated by visual inspection of residuals [27].

Results

Experiment I:

The effect of octopus and prey density on survival and growth

Although during experiment I casual cannibalism was observed (but not quantified), hatchling survival was not related to prey density ($p > 0.05$) or initial stocking density ($P > 0.05$) either through main terms or the interaction term ($P > 0.05$), and hatchlings from all treatment combinations had similar survival (Figure 1). The two way ANOVA on SGR resulted in a significant interaction between initial stocking density and prey density ($p < 0.05$), indicating that octopus growth under low and high density was affected in a different manner depending on the density in which prey were offered (Figure 2).

Mean growth rates expressed as SGR (%bw d⁻¹) for octopus in experiment I are shown in Table 2.

The two way ANOVA on ingestion rates showed that prey ($p < 0.05$)

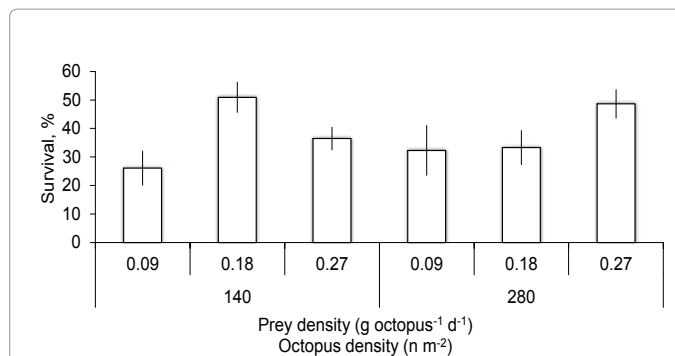


Figure 1: Survival (%) of two days old *O. maya* hatchlings cultured at two stocking densities (140 and 280 octopuses m⁻²) and three prey densities (0.09, 0.18 and 0.27 g shrimp octopus⁻¹ d⁻¹). Bars indicate standard deviations and letters indicate significant differences.

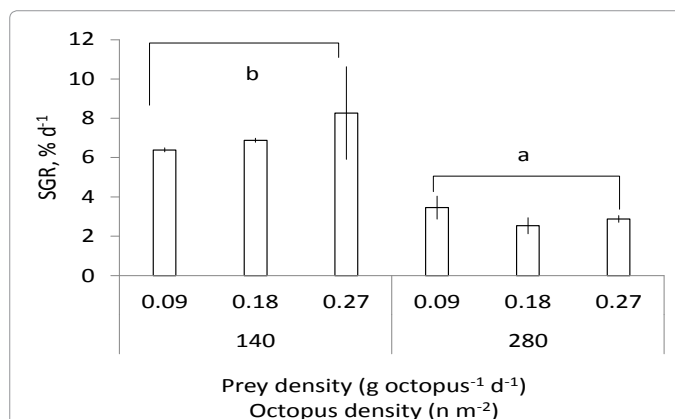


Figure 2: Specific growth rates (SGR, % bwd⁻¹) of two days old *O. maya* hatchlings cultured at two stocking densities (140 and 280 octopuses m⁻²) and three prey densities (0.09, 0.18 and 0.27 g shrimp octopus⁻¹ d⁻¹). Bars indicate standard deviations and letters indicate significant differences.

Stocking density	Prey density (g octopus ⁻¹ day ⁻¹)		
	0.09	0.18	0.27
140	6.39 ± 0.1	6.89 ± 0.1	8.26 ± 2.35
280	3.46 ± 0.58	2.54 ± 0.40	2.87 ± 0.17

Table 2: SGR (% bw d⁻¹) of *Octopus maya* hatchlings (2 days old) after 30 days in experiment I using different stocking (number of hatchlings m⁻²) and prey density (number of prey octopus⁻¹ d⁻¹) treatments. Values are mean ± SD; $n = 3$ in all cases.

and initial stocking density ($p < 0.05$) independently affected the IR_{ipt} %, and no significant effect was found in the interaction ($P > 0.05$) (Figure 3). Mean ingestion rate as percentage of food provided per tank (IR_{ipt}) showed a negative relationship to prey density, with higher values as prey density decreased for both low and high initial stocking densities (Figure 3A). Ingestion rate expressed in this manner was also higher in treatments amongst all treatments with high than with low octopus density (Figure 3B).

Food conversion index (FCI_i) varied with prey density, but did so in a different way depending on octopus initial density ($p < 0.01$). Whilst FCI_i in all treatments with low stocking density were statistically similar amongst each other, values increased with prey density amongst those treatments with high initial octopus density (Figure 4A). In contrast a reduction on GGE_i % was observed with octopus density, with high values on animals maintained at lower than higher densities (Figure 4B).

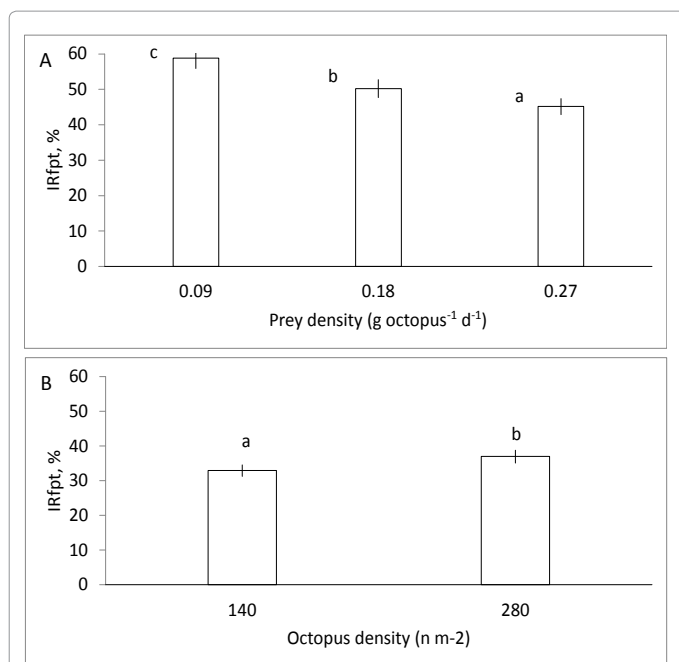


Figure 3: Ingestion rate as percentage of food ingested (IRfpt, %Fpt) of two days old *O. maya* hatchlings cultured for 30 days at two stocking densities (140 and 280 octopuses m⁻²) and three prey densities (0.09, 0.18 and 0.27 g shrimp octopus⁻¹ d⁻¹). Mean ± standard deviations; letters indicate significant differences P < 0.05.

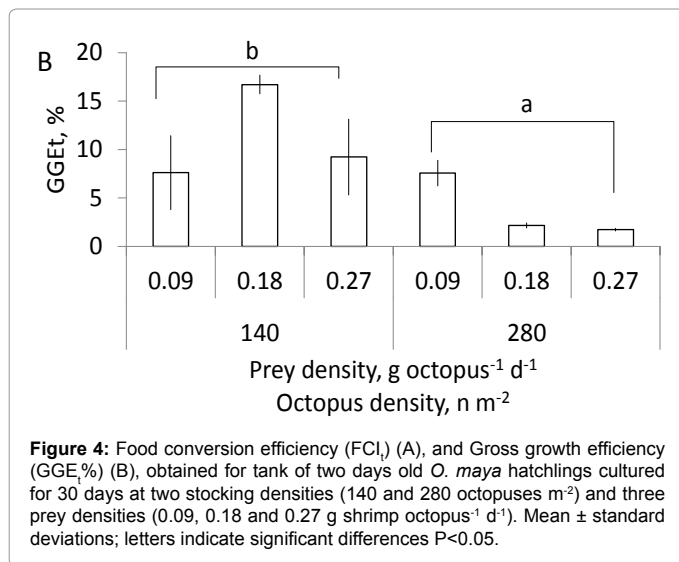


Figure 4: Food conversion efficiency (FCI) (A), and Gross growth efficiency (GGE, %) (B), obtained for tank of two days old *O. maya* hatchlings cultured for 30 days at two stocking densities (140 and 280 octopuses m⁻²) and three prey densities (0.09, 0.18 and 0.27 g shrimp octopus⁻¹ d⁻¹). Mean ± standard deviations; letters indicate significant differences P < 0.05.

Experiment II

The effect of prey density on growth of individualized *O. maya* hatchlings.

Prey density did not have a significant effect on growth (P > 0.05), and octopus fed with all three prey densities gained wet weight in a similar way (Figure 5). Survival of individualized animals was 60, 80 and 67% for treatments with 0.09, 0.18 and 0.27 g prey octopus⁻¹ d⁻¹.

Ingestion rate as percentage of food provided per animal (IR_{fpa}, %Fpa) showed a negative relation with prey density (p < 0.001), with the highest ingestion rate at the lowest prey density (0.09 g prey octopus⁻¹

d⁻¹), but statistically similar values for 0.18 and 0.27 g prey octopus⁻¹ d⁻¹ (Figure 6). The Food Conversion Index (FCI) and GGE_p, % did not show changes according to prey density (p > 0.05) with a mean value of 11.6 ± 8.25 and 11.9 ± 6.06 for animals fed three prey densities, respectively (Figure 7).

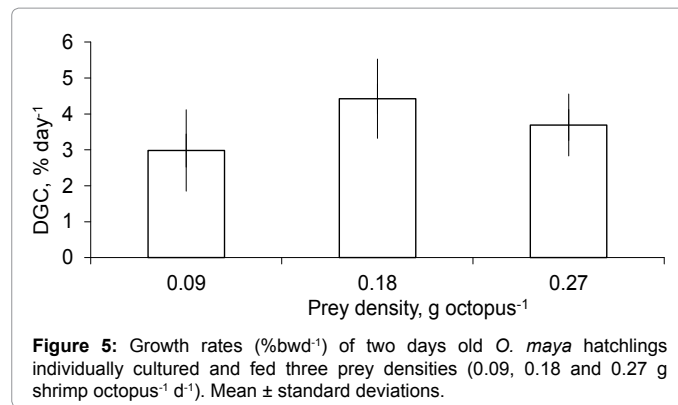


Figure 5: Growth rates (%bw d⁻¹) of two days old *O. maya* hatchlings individually cultured and fed three prey densities (0.09, 0.18 and 0.27 g shrimp octopus⁻¹ d⁻¹). Mean ± standard deviations.

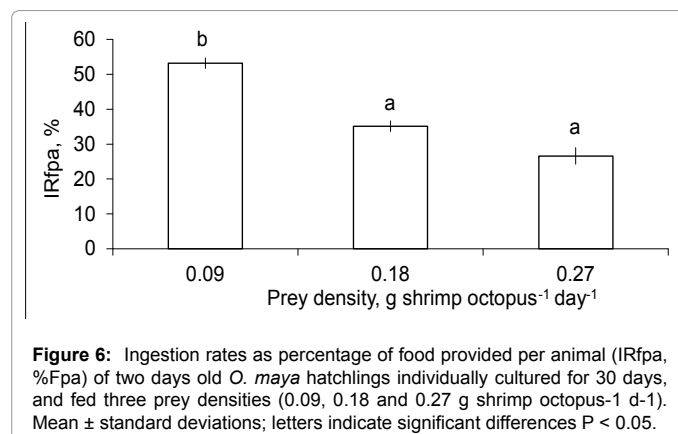


Figure 6: Ingestion rates as percentage of food provided per animal (IRfpa, %Fpa) of two days old *O. maya* hatchlings individually cultured for 30 days, and fed three prey densities (0.09, 0.18 and 0.27 g shrimp octopus⁻¹ d⁻¹). Mean ± standard deviations; letters indicate significant differences P < 0.05.

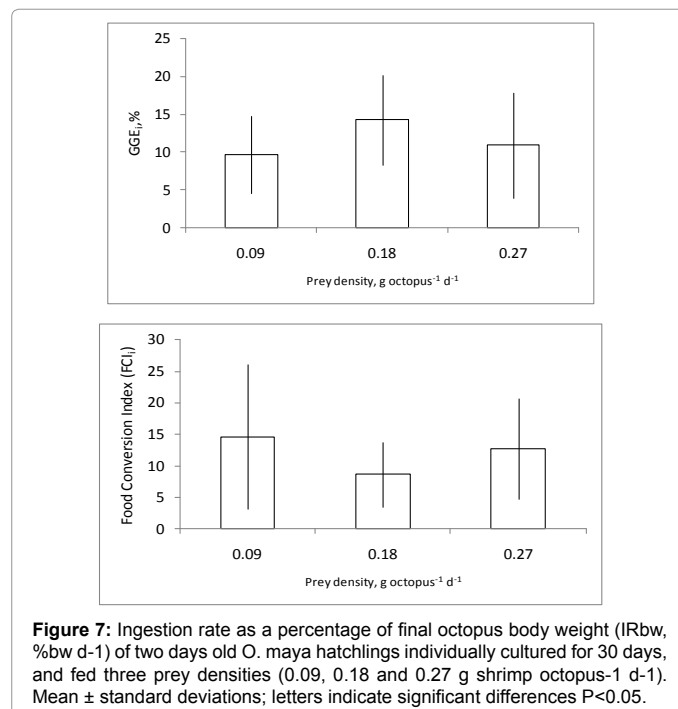


Figure 7: Ingestion rate as a percentage of final octopus body weight (IRbw, %bw d⁻¹) of two days old *O. maya* hatchlings individually cultured for 30 days, and fed three prey densities (0.09, 0.18 and 0.27 g shrimp octopus⁻¹ d⁻¹). Mean ± standard deviations; letters indicate significant differences P < 0.05.

Experiment III

Moderate cannibalism was observed during this experiment. Nevertheless, hatchling survival was similar amongst densities from 25 to 75 octopus's m⁻² (mean survival: 58 ± 2.93%). Contrary, survival decreased in octopus stocked at 125 hatchlings m⁻² (31%; Table 3). Final octopus weight ranged between 0.41 and 0.58 g, with low values for animals stocked at 125 octopuses m⁻² and high values for animals maintained at 25 octopuses m⁻², however, the negative relationship between growth and stocking density was not linear. Density affected the SGR (% day⁻¹) values with high values in animals maintained in low density in comparison to that obtained in the highest density (Table 3).

The high variability on wet weight at the end of the experiment did not allow a conventional statistical analysis. For that reason, GAM was applied to analyze the effect of animal density on final wet weight obtained during the experiment (Figure 8). The model with d.f.=3 had a lower AIC value than that with d. f.=2 (-1549.0, -1538.8, respectively) and proved to explain significantly more variability in final biomass ($p < 0.001$). The estimated variance in this model was $\sigma^2 = 0.01$ ($n = 854$), and only a 25.5% of the total deviance was explained, nevertheless indicating large amount of unexplained variation in the data. The smoothing term was significant ($p < 0.001$) indicating an important effect of density on final body octopus weight (Figure 8). The estimated intercept in the model was 0.69 ± 0.003 (\pm standard error.) and it was significantly different from 0 ($p < 0.001$). Thus, octopuses at an initial density of 50 octopuses m⁻² are predicted to weigh 0.42 g after 30 days of culture, whereas those at an initial density of 25 octopuses m⁻² are predicted to weigh of 0.59 g in the same period of time (Table 4) [27].

	Animals m ⁻²			
	25	50	75	150
Initial wet weight, g	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.02
N ^a			500	
Final weight, g	0.58 ± 0.02	0.53 ± 0.19	0.4 ± 0.11	0.41 ± 0.18
N ^b	3	3	3	1
N ^c	150	150	150	150
Days	30	30	30	30
SGR, % day ⁻¹	±	±	±	±
Survival, %	61 ± 5	55 ± 7	58 ± 9	31

- a. Number of hatchlings weighed at the beginning of the experiment
- b. Number of tanks of each experimental density
- c. Number of octopuses weighed at the end of the experiment per treatment

Table 3: Effects of animal density on growth and survival of *O. maya* hatchlings cultured during the first 30 days after hatch on 8m² dark tanks. Values as mean ± SE ±.

Density Animal m ⁻²	Smoother value	Predicted biomass (g)
25	0.08	0.59
	0.06	0.56
	0.04	0.53
	0.02	0.50
	0	0.48
50	-0.02	0.45
	-0.04	0.42
	-0.06	0.40
	-0.08	0.37

Table 4: Predicted biomass (g) of juvenile *O. maya* after 30 days of culture at different initial density (ind. /m²). Biomass values were estimated as: (smoother value + estimated intercept)².

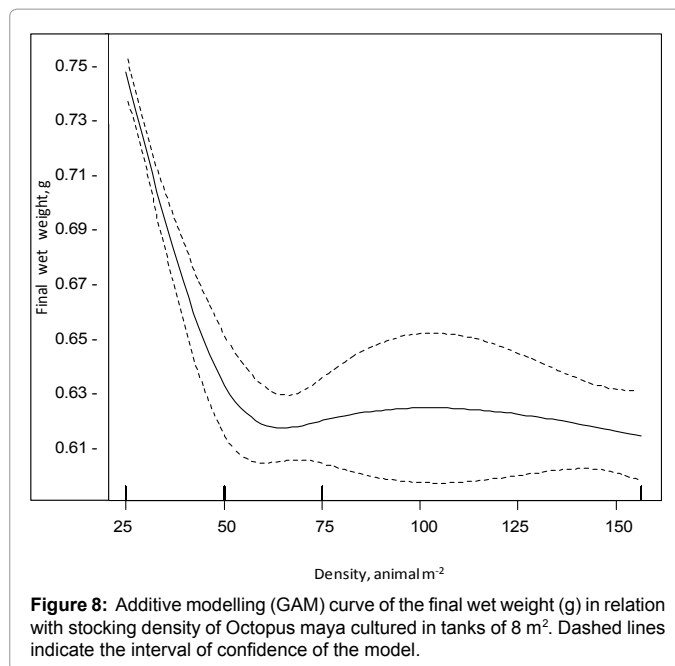


Figure 8: Additive modelling (GAM) curve of the final wet weight (g) in relation with stocking density of *Octopus maya* cultured in tanks of 8 m². Dashed lines indicate the interval of confidence of the model.

Discussion

Experiments I and II:

Results obtained in the present study showed that the relationship between mortality and growth rates had a different behavior depending on combinations between octopus and prey density; survival was not affected by prey or octopus density while a low growth was registered on animals maintained on high stocking density, independently of prey density.

Marquez *et al.* [18] suggests a type III functional response for cuttlefish hatchlings preying on live mysids, since a peak is reached and afterwards the increase in prey density did not lead to higher consumptions. This occurred in the present study when octopus density changed in animals cultivated in groups; animals maintained in low densities showed an increment on prey consumption, while animals cultured at high density showed an inverse reduction on prey consumption according with prey density increment. In individualized animals, ingestion rate increased constantly with prey density suggesting that, when octopuses had no school pressures, prey consumption increases in direct relation to prey density provoking higher growth rates.

During experiment I, casual cannibalism was observed although not quantified. Cannibalism has been reported for several cephalopod species such as *O. vulgaris* [28] and *Loligo vulgaris* [29]. The higher stocking densities increase the rates of encounters between hatchlings, which most likely increases cannibalism. In general, the behavior of the victim (as that of the aggressor) can be the reason for cannibalism and as behavior often is density-related, the rates of cannibalism are often related to the number of encounters [30]. Results from experiment I suggest that *O. maya* hatchlings do not support culture densities higher than 140 octopuses m⁻², even when ideal live prey is provided. Not only cannibalism is likely enhanced, but growth was markedly reduced when animals were cultivated at 280 octopuses m⁻², probably due to stress and competition for territory or food [31]. In fact, it was frequent to observe that in this high density, hatchlings chasing each other, or

trying to steal a shrimp captured by other octopuses, which probably generated great stress and the loss of considerable amounts of energy. Boal et al. [32] studied the effects of crowding in *S. officinalis* with two different culture densities, and suggested that cuttlefish cultured at lower densities were less stressed, and because of this ate and grew more, while the ones cultured at higher densities showed signs of stress.

S. officinalis presents a much less cannibalistic behaviour, are very tolerant to high stocking densities [14,33], and most likely can be cultured at higher densities than *O. maya* during the first part of their life cycle. Although maximum densities of about 1000 cuttlefish m^{-2} for small juveniles (<3 cm mantle length, ML) were suggested by Forsythe et al. [34] it is necessary to consider differences between benthic and nektonic species; cuttlefish densities should consider the volume while octopus species should be considered in relation with the area of the benthic environment.

Results from the IR_{pt} % clearly indicate that hatchlings cultured at 140 octopuses m^{-2} were less stressed and fulfilled their nutritional requirements before and more efficiently than those cultured at 280 octopuses m^{-2} . This is explained by the gradual decrease between all treatments in IR_{pt} % with increasing prey density. Contrary to results obtained in the present study, Forsythe et al. [34] reported that the effects of culture density in juvenile (<1.5 g) *S. officinalis* (100 and 400 cuttlefish m^{-2}) had no statistical significance in food consumption or growth rates, but suggests that stocking densities of 400 cuttlefish m^{-2} may be approaching levels that affect feeding and growth. Warnke [35] reported that group reared cuttlefish ate more and grew larger (up to 9%) compared to others placed in isolation, while Dickel [36,37] indicated that *S. officinalis* reared in isolation, or in poor environments (e.g. simple tank with no substrate or hiding places) grew less than others placed in groups, or in enriched environments (e.g. tanks with shells, sand and shelters). Similarly, [38] indicated that *S. officinalis* can be cultured in isolation only for short-term experiments. The comparison between IR from experiments I and II in the present study does not support these findings, since octopuses placed in isolation ate more than those cultured in groups. Again, this could be due to the fact that *O. maya* is more territorial than *S. officinalis*, and therefore isolation provides less stress and the absence of competition for food or territory. Domingues et al. [39] also observed that isolated cuttlefish inked more and appeared to be more stressed than group cultured ones, although this was not reflected in growth or survival.

Experiment III:

Results obtained in small tanks suggest that for *O. maya* culture densities lower than 140 octopuses m^{-2} must be used during first 30 days. For that reason experiment III tested densities between 25 to 75 octopus m^{-2} . According to the GAM model used, a density between 25 to 50 octopuses m^{-2} is adequate to culture *O. maya* hatchlings on 8 m^2 tanks, since with such densities the higher final weight and survival were obtained. Between 25 and 75 octopus m^{-2} , survival was not affected, suggesting that in this stock density range cannibalism should not be an important factor. As During this part of the study hatchlings were fed crab paste and *Artemia* adults during first 15 days followed by other 15 days with only crab paste and using a ratio of 150% of octopus wet weight [5]. Apparently, those rations and diets covered the nutritional requirements of hatchlings cultured between 25 to 75 octopus m^{-2} , and can be recommended as culture conditions for *O. maya* juveniles in experimental pilot scale. Lower growth at higher densities was reported for the cuttlefish, *Sepia officinalis* [14], while Otero reported differences in food conversion and growth for juveniles of *Octopus vulgaris* stocked at 10 and 20 Kgm^{-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. Domingues et al. [12] also reports different food conversions for juvenile octopus, but reported no differences in growth rates with initial densities between 4 and 16 animals m^{-2} . These authors suggest that initial density should not be higher than 10 $Kg m^{-3}$ for *O. vulgaris* juveniles.

The statistical model used here also showed the necessity to evaluate octopus growth rate in a different form, because in it was included variability. Variability of growth rates on cephalopods has been widely recognized and, as in the present study, must be considered in all studies where octopus culture needs to be applied [40-45]. Variability is the key of aquaculture, because through variability it is possible design process to enhance survival, reducing at the same time, heterogeneity of cultured population. Considering this, *O. maya* juveniles production has incorporated the variability evaluation every 30 days of culture to separate animals with different sizes, delivering higher survival and growth rates at pilot scale culture conditions.

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References

1. Voss GL, Solís-Ramírez M (1966) *Octopus maya*, a new species from the Bay of Campeche, México. Bulletin of Marine Science 16: 615-625.
2. Solís M (1998) Aspectos biológicos del pulpo *Octopus maya* Voss y Solís. Contribuciones de Investigación Pesquera, Instituto Nacional de la Pesca 7: 1-38.
3. Rosas C, Gallardo P, Mascaró M, Caamal-Monsreal C, Pascual C (2014) *Octopus maya*. In: Cephalopod Culture. Edited by Iglesias J, et al. Dordrecht: Springer Science+Business Media: DOI 10.1007/1978-1094-1017-8648-1005_1020.
4. Domingues P, López N, Muoz JA, Maldonado T, Gaxiola G, Rosas C (2007) Effects of an artificial diet on growth and survival of the Yucatan octopus, *Octopus maya*. Aquaculture Nutrition 13: 1-9.
5. Rosas C, Tut J, Baeza J, Sánchez A, Sosa V, Pascual C, Arena L, Domingues P, Cuzon G (2008) Effect of type of binder on growth, digestibility and energetic balance of *Octopus maya*. Aquaculture 275: 291-297.
6. DeRusha R, Forsythe JW, DiMarco FP, Hanlon RT (1989) Alternative diets for maintaining and rearing cephalopods in captivity. Lab. Anim. Sci 39: 312.
7. Hanlon RT, Forsythe JW (1985) Advances in the laboratory culture of Octopuses for biomedical research. Laboratory Animal Science 35: 40.
8. Van Heukelem WF (1976) Growth, bioenergetics and life span of *Octopus cyanea* and *Octopus maya*. PhD Thesis, Univ. Hawaii.
9. Van Heukelem WF (1977) Laboratory maintenance, breeding, rearing and biomedical research potential of the Yucatan octopus (*Octopus maya*). Lab. Anim. Sci 27: 852-859.
10. Van Heukelem WF (1983) *Octopus maya* In: Cephalopod Life Cycles. Academic Press, London 1: 311-323.
11. Forsythe JW, Van Heukelem WF (1987) Growth. In: Cephalopod life cycles. Edited by Boyle PR. Comparative Reviews. London: Academic Press 2: 135-155.
12. Domingues P, Garcia S, Garrido D (2010) Effects of three culture densities on growth and survival of *Octopus vulgaris*. Aquaculture International 18: 165-174.
13. Rosas C, Cuzon G, Pascual C, Gaxiola G, López N, Maldonado T, Domingues P (2007) Energy balance of *Octopus maya* fed crab and artificial diet. Marine Biology 152: 371-378.
14. Sykes A, Domingues P, Andrade JP (2014) *Sepia officinalis* In: Cephalopod Culture. Edited by Iglesias J, Fuentes L, Villanueva R. Dordrecht: Springer Science + Business Media 175-205.
15. Villanueva R, Sykes A, Vidal AT, Rosas C, Nabhitabhata J, Fuentes L, Iglesias J (2014) Current Status and Future Challenges in Cephalopod Culture. In:

- Cephalopod culture. Edited by Iglesias J, Fuentes L, Villanueva R. New York: Springer Science+Business Media: 479-489.
16. Borer KT, Lane CE (1971) Oxygen requirements of *Octopus briareus* Robson at different temperatures and oxygen concentrations. *J Exp Mar Biol Ecol* 7: 263-269.
 17. Koueta N, Boucaud-Camou E (1999) Food intake and growth in reared early juvenile cuttlefish *Sepia officinalis* L. *Mollusca Cephalopoda*. *J Exp Mar Biol Ecol* 240: 93-109.
 18. Marquez L, Caballos M, Domingues P (2007) Functional response of early stages of the cuttlefish *Sepia officinalis* preying on the mysid *Mesopodopsis slabberi*. *Mar Biol Res* 3: 462-467.
 19. Correia M, Domingues P, Sykes A, Andrade JP (2005) Effects of culture density on growth and broodstock management of the cuttlefish, *Sepia officinalis* (Linnaeus, 1758). *Aquaculture* 245: 163-173.
 20. Marquez L, Quintana D, Almansa E, Navas JI (2007) Effects of visual conditions and prey density on feeding kinetics of paralarvae of *Octopus vulgaris* from a laboratory spawning. *J. Mollus. Stud* 73: 117-121.
 21. Domingues P, Márquez L (2010) Effects of culture density and bottom area on growth and survival of the cuttlefish *Sepia officinalis* (Linnaeus, 1758). *Aquacult Int* 18: 361-369.
 22. Moguel C, Mascaró M, Avila-Poveda O, Caamal C, Sánchez A, Pascual C, Rosas C (2010) Morphological, physiological, and behavioural changes during post-hatching development of *Octopus maya* (Mollusca: Cephalopoda) with special focus on digestive system. *Aquatic Biology* 9: 35-48.
 23. Vidal E, DiMarco FP, Wormuth JH, Lee PG (2002) Influence of temperature and food availability on survival, growth and yolk utilization in hatchling squid. *Bull mar Sci* 71: 915-931.
 24. Wigglesworth JM, Griffith DRW (1994) Carbohydrate digestion in *Penaeus monodon*. *Mar. Biol* 120: 571-578.
 25. Zar JH (1999) *Biostatistical Analysis*. New Jersey: Prentice-Hall.
 26. Underwood AJ (1997) *Experiments in ecology: Their logical design and interpretation using analysis of variance*, Cambridge University Press, Cambridge.
 27. Montgomery DC, Peck EA (1992) *Introduction to Linear Regression Analysis* (2nd edtn). New York: John Wiley.
 28. Vaz-Pires P, Seixas P, Barbosa A (2004) Aquaculture potential of the common octopus *Octopus vulgaris* (Cuvier, 1797) a review. *Aquaculture* 238: 221-238.
 29. Summer WC, McMahon JJ (1970) Survival of unfed squid, *Loligo pealei* in an aquarium. *Biol Bull* 138: 389-396.
 30. Fox LR (1975) Cannibalism in natural populations. *Ann Rev Ecol Syst* 6: 87-106.
 31. Ibañez C, Chong JV (2008) Feeding ecology of *Enteroctopus megalocyathus* (Cephalopoda: Octopodidae) in southern Chile. *J Mar Biol Ass UK* 88:793-798.
 32. Boal JG, Hylton RA, Gonzales SA, Hanlon RT (1999) Effects of crowding on the social behaviour of cuttlefish (*Sepia officinalis*). *Contemporary Topics in Laboratory Animal Science* 38: 49-55.
 33. Forsythe JW, Lee G, Walsh L, Tara C (2002) Effect of crowding on growth of the European cuttlefish, *Sepia officinalis* Linnaeus, 1758 reared at two temperatures. *J Exp Mar Biol Ecol* 269: 175-183.
 34. Forsythe JW, DeRusha RH, Hanlon RT (1994) Growth, reproduction and life span of *Sepia officinalis* (Cephalopoda: Mollusca) cultured through seven consecutive generations. *J Zool* 233: 175-192.
 35. Warnke K (1994) Some aspects of social interaction during feeding in *Sepia officinalis* (Mollusca: Cephalopoda) hatched and reared in the laboratory. *Vie et Millieu* 44: 125-131.
 36. Dickel L (1999) Effets de l'expérience précoce sur la maturation des capacités mnésiques au cours de l'ontogenèse post-embryonnaire chez la seiche (*Sepia officinalis*). *Ann Fond Fyssen* 14: 86-94.
 37. Dickel L, Boal JG, Budelmann BU (2000) The effect of early experience on learning and memory in cuttlefish. *Develop Psychobiol* 36: 101-110.
 38. DeRusha RH, Forsythe JW, DiMarco FP, Hanlon RT (1989) Alternative diets for maintaining and rearing cephalopods in captivity. *Laboratory Animal Science* 39: 306-312.
 39. Domingues P, Poirer R, Dickel L, Almansa E, Sykes A, Andrade JP (2003) Effects of culture density and live prey on the growth and survival of juvenile cuttlefish, *Sepia officinalis*. *Aquacult Int* 11: 225-242.
 40. Pecl G, Steer MA, Hodgson K (2004) The role of hatchling size in generating the intrinsic size-at-age variability of cephalopods: extending the Forsythe hypothesis. *Mar Freshw Res* 55.
 41. Doubleday Z, Semmens JM, Pecl G, Jackson G (2006) Assessing the validity of stylets as ageing tools in *Octopus pallidus*. *J Exp Mar Biol Ecol* 338: 35-42.
 42. Leporati S, Pecl GT, Semmens JM (2007) Cephalopod hatchling growth: the effects of initial size and seasonal temperatures. *Mar Biol* 151: 1375-1383.
 43. André J, Grist EPM, Semmens JM, Pecl G, Segawa S (2009) Effects of temperature on energetics and the growth pattern of benthic octopuses. *J Exp Mar Biol Ecol* 374: 167-179.
 44. Briceño-Jacques F, Mascaró M, Rosas C (2010) GLMM-based modelling of growth in juvenile *Octopus maya* siblings: does growth depend on initial size? *ICES J Mar Sci* 67: 1509-1516.
 45. Briceño-Jacques F, Mascaró M, Rosas C (2010) Energy demand during exponential growth of *Octopus maya*: exploring the effect of age and weight. *ICES J Mar Sci* 67: 1501-1508.