Effects of a dry pelleted diet on growth and survival of the Yucatan octopus, *Octopus maya*

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Abstract

The effect of a dry pelleted diet on growth of the Yucatan octopus (*Octopus maya*) was determined and compared with crab diet (*Callinectes* spp). Two groups of 15 wild collected animals were used. Octopuses were placed in isolation, in a flow-through system composed of 30 circular tanks (80 L), with a bottom of 40 cm in diameter and a water depth of 80 cm. Experiment lasted 40 days, and octopuses were weighed every 10 days to determine growth rates. Octopuses were fed between 7% and 10% body weight (BW) per day, twice a day at 9:00 h and 17:00 h. Uneaten food was removed after 4 h in the tanks and weighed, to determine food ingestion and conversion. No mortality as a result of natural causes was observed. The 15 octopuses fed on frozen crabs and the 15 fed on the artificial diet had similar weights ($P > 0.05$) at the start of the experiment, of 486.2 ± 214.8 and 472.5 ± 228.3 g, respectively. At the end of the experiment, octopuses fed on frozen crabs weighed 1466.5 ± 484.0 g, and were significantly larger ($P < 0.05$), than octopuses fed on artificial diets (438.9 ± 202.6 g). Growth rates for the experiment were of 3.3 ± 0.2 and –0.0 ± 0.3% BW per day, for octopuses fed frozen crabs and the artificial diet, respectively. The artificial diet did not promote growth, but animals did not loose weight and more important, ate regularly all the food supplied, with feeding rates higher than reported in the literature for prepared diets. This makes *O. maya* a good research animal for the development of artificial diets for cephalopods.

KEY WORDS: artificial diets, growth, nutrition, octopus, *Octopus maya*

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Introduction

Cephalopods are characterized by life cycles as short as 6–9 months (Okutani 1990; Domingues *et al.* 2001a,b,2002), but the majority of the species can live between 1 and 2 years (Mangold 1983). All cephalopods are carnivorous (Boucher-Rodoni *et al.* 1987; Okutani 1990; Lee 1994), active predators (Boletzky & Hanlon 1983) and an important fishery, being consumed by humans in many regions around the world (Lee *et al.* 1998). They present high metabolism, which contributes to the fast growth rates that vary between 3% and 10% body weight per day (BW) in adults (Amaratunga 1983; Lee 1994; Lee *et al.* 1991,1998; Domingues *et al.* 2002; Sykes *et al.* 2003), and higher than 10% BW per day in the early stages of the life cycle (Domingues *et al.* 2001a,b, 2002,2003,2004). Feeding rates (FR) depend on water temperature and can be as high as 50% BW per day (Mangold 1983; Boucher-Rodoni *et al.* 1987; Domingues *et al.* 2001a,2003,2004) and food conversions (FC) (>50%) are among the highest in animals (Richard 1975; Amaratunga 1983; Van Heukelem 1976,1977; Mangold 1983; Forsythe & Van Heukelem 1987). Choe (1966) reports conversion rates that vary between 9% and 71% BW per day, averaging 38.7%, while Domingues *et al.* (2001a,2004) reports FC between 35% and 50%. Because of their protein metabolism, their requirement in proteins and amino acids is high (Lee 1994), and they are unique among the poikilotherms (Forsythe & Van Heukelem 1987; Lee *et al.* 1998).

Appropriate and inexpensive diets are basic requirements for the success in commercial aquaculture (Chen & Long 1991). The use of prepared diets replacing natural diets can reduce production costs by 40% initially; afterwards, further reductions can be obtained (Lee 1994). The dependence on natural prey has prevented large-scale culture of cephalopods (O'Dor & Wells 1987; Lee *et al.* 1991; Domingues 1999). Another limitation that has prevented large-scale culture until the present days is the existence of very few alternative diets that can be successfully used to culture cephalopods (DeRusha *et al.* 1989; DiMarco *et al.* 1993; Lee 1994; Domingues *et al.* 2005). Some cephalopod species, with direct egg development, such as *Sepia officinalis* or *Octopus maya* have been maintained with artificial diets (Castro 1991; Lee *et al.* 1991; Domingues 1999; Domingues *et al.* 2005). Nevertheless, growth obtained was moderate compared with natural diets.

Octopus maya is an endemic species from the Yucatan peninsula (Voss & Solis 1966); according to Solis (1967), its distribution ranges from the Campeche Bay, in the North of the Yucatan peninsula, to Isla Mujeres. Nevertheless, the distribution range has recently been widened in the north limit, to Ciudad del Carmen, Campeche (Solis-Ramirez 1994,1997). This species easily adapts to laboratory conditions, and accepts dead prey or prepared diets immediately after being born (Domingues, personal observations). *Octopus maya* has been cultured in the laboratory (Solis 1967; Van Heukelem 1976,1977; Hanlon & Forsythe 1985; DeRusha *et al.* 1989), up to four (Hanlon & Forsythe 1985) or five (Van Heukelem 1983) generations. This species has fast growth rates up to 8% BW per day, because of high FR and FC, which vary between 30% and 60% (Hanlon & Forsythe 1985); Van Heukelem (1976,1983) obtained FC of 40% for this species. *Octopus maya* can grow up to 1 kg in 4 months and upto 3 kg in 9 months, at 25 °C. This species reaches maximum weight at 9 months (Van Heukelem 1983). Among the species of octopuses with large eggs and direct embryonic development, *O. maya* is the one that presents highest growth rates, reaching 5.7 kgs with average growth rates of 4.1% BW per day, and is one of the better candidates for commercial aquaculture in high culture densities, after *O. bimaculoidis* and *O. digueti* (Hanlon & Forsythe 1985). *O. maya* was chosen for these experiments because: (i) octopuses are highly adaptable for laboratory conditions (Boletzky & Hanlon 1983); (ii) they accept dead or artificial feeds early in the life cycle, compared with other cephalopods; and (iii) *O. maya* has eggs with direct development, contrary to the majority of the octopuses species that has planctonic larvae (Boletzky 1974), making them easier to culture in the laboratory.

The objective of this study was to determine the effects of an artificial diet on growth and survival. For this propose, an improved commercial shrimp diet was compared with fresh crab (*Callinectes* sp.), as octopuses prefer crabs to any other type of prey (Castro & Guerra 1990).

Materials and methods

Animals, diets and growth

A total of 30 animals were used: 15 were fed the artificial diet and 15 were fed frozen crabs. Both groups had similar weights at the start of the experiment: 486.2 ± 214.8 and 472.5 ± 228.3 g, diet and crab, respectively. The diet was prepared in the laboratory, using as a base a shrimp commercial feed with high protein content and squid paste (Purina@ 51%; Table 1). The CPSP 90% (hydrolysed protein concentrate with 86.3% protein content) with lot no. 050201 was bought from 'Cooperative de Tratment des Produits de la Peche' (CTPP), France. The ROVIMIX® STAY-C® 35 – code D4 8304 4 lot PD9 0769, came from ROCHE®, France. The vitamin mix (0302) and the mineral mix (C-22/94-2) were bought from DIBAQ®. The squid used was a *Loliguncula brevis*, captured in the Gulf of México, and bought fresh in a local market. The components of the diet were mixed with squid meat (previously defrosted) blended separately. This delivered a semi-humid diet with high cephalopod scent and flavour, which was stored at 6 °C. The diet was elaborated on a daily basis. Octopuses were maintained in individual tanks, in a flow-through culture system, which was composed of 30 circular tanks (80 L), with bottom of 40 cm in diameter and water depth of 80 cm, approximately. Each tank was covered with a green solid net, sowed around the top of the tank, to prevent octopuses escaping. Natural filtered sea water (5 μm)

Table 1 Composition (g kg^{-1}) of the artificial diet used to feed *Octopus maya* during the experiment. (i) fish-soluble protein concentrate, (ii) fresh squid, (iii) Rovimix Stay C is L-ascorbyl-2-monophosphate, from DSM (Dutch State Mines, earlier Roche Vitamins) and (iv) from DSM, for shrimp

Ingredients	g kg^{-1}
Shrimp diet (Purina@ 51%)	550
CPSP@, 90% (CTPP)[1]	50
Fresh Squid (*Loliguncula brevis*)[2]	200
Soybean lecitin	150
Rovimix stay C (Roche@)3	20
Mineral and vitamins mix (Dibaq@)4	30

Abbreviations: CPSP, hydrolysed protein concentrate with 86.3% protein content; CTPP, Cooperative de Tratment des Produits de la Peche. CPSP (registered name of a product of CTTP) comes with two concentrations of protein. CPSP90 has about 85% proteins.

flowed at 120 L h^{-1}, allowing a complete renewal in each tank every 40 minutes. Temperature (°C) was measured twice a day, and salinity (g L^{-1}) every 2 days. A piece of PVC pipe (±35-cm long, 10-cm diameter) was placed in each tank to provide shelter for the octopuses.

The experiment lasted 40 days, and octopuses were individually weighed every 10 days to determine growth rates and to correct the FR. Octopuses were fed twice a day (0900 and 1700 h) between 7% and 10% octopuses dry weight (DW) per day for both diets (Van Heukelem 1983; Domingues *et al*. 2001b, 2002, 2003, 2004). The growth rate was determined by subtracting the weight at the start from the weight at the end of each weighing period (t).

Instantaneous growth rate (IGR) was determined as: IGR (% BW per day) = [(lnW2–lnW1)/t]*100, where W2 and W1 are the final and initial wet weights (WW) of the octopuses, ln the natural logarithm and t the number of days of the time period.

Food ingestion

After 4 h in the tanks, uneaten food was removed, dried and weighed, to determine ingestion and FC. Lixiviation and water absorption of the food was calculated as the difference between food DW before and after 4 h of soaking. The correlation factor was 1.42 ± 0.04, meaning that a piece of diet that weighed 1 g before being fed to the octopuses, weighed 1.42 g after 4 h in the tanks.

Faeces were collected once a day when present in the tanks, except early in the morning. Faeces were immediately dried in the oven for determination of water content, and then stored in a dry place, for posterior analysis (calorimetric value and biochemical composition). The FR was calculated as: (FR) (% BW per day) = (FI/average W(t))*100, where FI is the food ingested in a period of time (t), W(t) is the average WW of the octopus during the period of time (t) in days.

FC was calculated as: (FC) = (W2–W1)/FI*100; W2–W1 is the difference in living weight and FI is the food ingested in a period of time.

The absorption efficiency of the diet (AE) was determined, by using faeces recovered from octopuses fed each diet. Faeces were dried at 60 °C until constant DW; part of the dry faeces was used to determine energy content (caloric value) and part to determine ash content. The AE was determined using the equation: AE = [(DWAF/DWd–DWAF/DWf))/(1–DWAF/DWf)DWAF/DWd)] × 100, where DWAF/DWd is the ratio between the DW ash-free and the DW of the diet, and DWAF/DWf is the ratio between the DW ash-free and the DW of the faeces. The energy absorbed (joules/g DW per day) by octopuses was calculated as: AbE = AE * IR * FE where IR is the ingestion rate (g DW per day) and FE is the food energy content expressed as joules g^{-1} DW. Food energy content was determined with a calorimetric pump (Parr@) calibrated with benzoic acid.

Biochemical composition

At the end of the experiment, five octopuses of each group were sacrificed for determination of water and ash content, and biochemical composition. Samples of crabs, faeces and the artificial diet were also dried to determine water and ash content, as well as biochemical composition. Water content was determined at 60 °C until constant weight. Ash content was determined by burning dry samples in an oven, at 400 °C for 4 h (AOAC method 942.05). Lipids (ethereal extract) were determined using the AOAC method 920.39; protein was determined using Kjeldahl method AOAC 984.13; crude was determined using the gravimetric method AOAC 962.09; free nitrogen extract was determined using the AOAC NMX-Y-097-1974 method. The amino acid auto analyser used was a Beckman System 6300, with amino acid analysis made according to AOAC official method 982.3 (AOAC 2000) method for all the amino acids, except for tryptophan, where alkaline hydrolysis was used, with the method AOAC 988.15 (AOAC 2000).

Statistics

Non-parametric Kruskal-Wallis ANOVA tests (Zar 1999) determined significant differences in FR, FC and IGR of octopuses fed on the two diets between weighing periods. Student's t-tests (Zar 1999) were used to compare differences in weight between octopuses fed on the two diets after weighing procedures.

Results

Throughout the experiment, water temperature varied between 28 ± 1 °C, and salinity between 36 ± 2 g L^{-1}. Type of diet affected the living weight of octopuses. Animals fed on crabs were significantly larger than those fed on the artificial diet (Fig. 1). After 10 days, octopuses fed on frozen crabs weighed 736.5 ± 302.9 g and were larger ($P = 0.005$) than the ones fed on the artificial diet (441.1 ± 205.7 g). At the end of day 20, octopuses fed on frozen crabs ($n = 9$) weighed 916.1 ± 401.0 g and were larger ($P = 0.005$) than the ones fed on the artificial diet ($n = 14$) that weighed 443.4 ± 217.9 g. After 30 days, octopuses fed on frozen

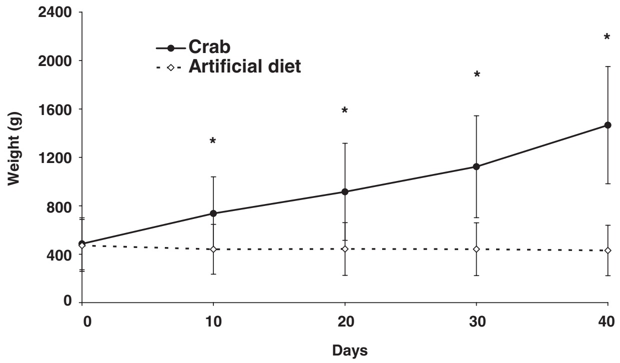

Figure 1 Growth of octopuses (gram of wet weight) fed either frozen crabs or an artificial diet during 40 days. Bars represent standard deviations; *asterisk* indicates values that are significantly different ($P < 0.05$) between the two groups, at the end of that weighing period.

crabs weighed 1123.0 ± 421.2 g and were larger ($P = 0.0001$) than the ones fed on the artificial diet that weighed 442.2 ± 218.2 g. At the end of the experiment, octopuses fed on frozen crabs weighed 1466.5 ± 484.0 g and were larger ($P = 0.0001$) than the ones fed on the artificial diet that weighed 431.3 ± 208.8 g (Fig. 1).

The growth rates based on WW (IGR = % BW per day) were higher ($P = 0.000$) for octopuses fed on the frozen crabs than those fed on the artificial diet. The IGR varied between 2.2% and 4.3% BW per day for animal fed on crabs while for octopuses fed artificial diet IGR was almost zero and practically constant during the entire experimental period (Fig. 2).

FR was always significantly higher in octopuses fed on crab than that observed in animals fed on the artificial diet ($P < 0.05$; Table 2), with the exception of the last 10 days of the experiment. A reduction in FR in both experimental groups was observed between days 30 and 40. FC was always significantly higher ($P < 0.05$) for animals fed on frozen crabs than for those fed on the artificial diet (Table 2).

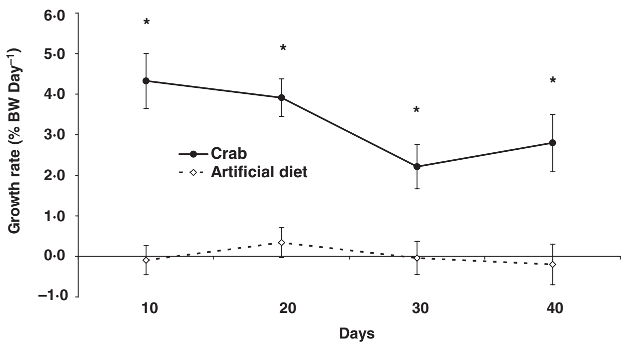

Figure 2 Growth rate (% BW per day) of octopuses fed frozen crabs or the artificial diet. Bars represent standard deviations; *asterisk* indicates values that are significantly different ($P < 0.05$) between the two groups, at the end of that weighing period.

Living weight, DW and energy content (caloric value) of octopuses fed on frozen crabs and those fed on the artificial diet were not significantly different. A mean DW value of 15.8 ± 1.7% BW was calculated for octopuses fed both diets. Table 3 shows ingestion rates (g afDW per day per gram DW octopus), absorption (joules per day g^{-1} DW) and assimilation efficiency (%) of *O. maya* fed on the artificial diet or frozen crab during the experiment.

Octopuses fed on crabs had the highest amount of protein (740 ± 21 g kg^{-1} of the DW), while the ones fed on the artificial diet had a concentration of protein of 659 ± 10 g kg^{-1}. Both diets had less protein (563 ± 21 for the frozen crab and 421 ± 24 g kg^{-1} for the artificial diet, in DW) compared with mantle composition of the octopuses. The artificial diet had a considerably higher lipid content (215 ± 12 g kg^{-1}) compared with frozen crab (47 ± 15 g kg^{-1}) (Table 4).

The overall amino acid composition of the artificial diet does not seem to be much different from the octopus mantle. Nevertheless, overall amino acid composition of *O. maya* used in this experiment was higher than the one of *Octopus vulgaris* juveniles (14 g WW) reported by Villanueva *et al.* (2004) (Table 5).

Discussion

Research on artificial diets for cephalopods has increased during the past decade, but requires further intensification; more financial resources, researchers and research centres need to be involved (Domingues 1999). DeRusha *et al.* (1989) tried to raise cuttlefish (*S. officinalis*) using adult artemia replacing mysids; Castro (1991) using artificial diets based on shrimp paste. Both, however, obtained poor growth and survival. At the start of the 1990s, research on artificial diets for cephalopods (cuttlefish and octopuses) was initiated at the National resource Center for Cephalopods, in Galveston, Texas (EUA) (Lee *et al.* 1991). The diets did not promote growth; afterwards, Castro *et al.* (1993) used a catfish fillet base that was accepted by cuttlefish, also with poor results. Then, a similar diet was enriched with proteins and other nutrients promoting moderate growth (Castro & Lee 1994). Finally, Domingues (1999) and Domingues *et al.* (2005) used similar diets, supplemented with proteins and amino acids for cuttlefish, with one (with the highest protein and amino acid composition) promoting moderate growth

Table 2 Feeding rate, both in dry weight (DW) and wet weight (WW) [% body weight (BW) per day] and food conversions (FC), also in DW and WW (%) for *Octopus maya* fed either an artificial diet or frozen crabs during 40 days

Days	10	20	30	40
Feeding rate (*DW*) (% BW per day)				
Frozen crabs	9.8 ± 1.2*	10.1 ± 1.1*	9.7 ± 2.1*	7.7 ± 1.6
Artificial diet	8.1 ± 0.4*	8.0 ± 0.4*	8.0 ± 0.4*	6.9 ± 0.5
Feeding rate (*WW*) (% BW per day)				
Frozen crabs	7.0 ± 0.9*	7.6 ± 0.8*	7.1 ± 1.6*	4.8 ± 2.0*
Artificial diet	2.8 ± 0.3*	2.7 ± 1.2*	2.6 ± 0.8*	2.4 ± 0.2*
Food conversions (*DW*) (%)				
Frozen crabs	44.0 ± 7.4*	38.8 ± 8.3*	24.1 ± 3.7*	38.4 ± 7.0*
Artificial diet	–0.8 ± 4.3*	4.2 ± 4.5*	0.3 ± 5.2*	–2.2 ± 7.3*
Food conversions (*WW*) (%)				
Frozen crabs	58.9 ± 10.1*	47.7 ± 11.4*	33.0 ± 5.0*	52.3 ± 9.5*
Artificial diet	–3.2 ± 12.3*	12.0 ± 12.9*	0.7 ± 15.0*	–6.3 ± 21.0*

*indicates the values that are significantly different ($P < 0.05$) between the two groups, at the end of that weighing period.

Table 3 Ingestion rates, absorption and assimilation efficiency of *Octopus maya* fed the artificial diet or the frozen crab during the experiment

	Crab diet	Artificial diet
Ingestion rate		
Grams afDW/day per gram DW octopus	0.047 ± 0.003	0.12 ± 0.002
Absorption		
Joules per day g^{-1} DW	764.8 ± 55.9	603.7 ± 10.8
Assimilation efficiency (%)	96 ± 0.002	23 ± 0.01

DW, dry

Table 4 Composition (g kg^{-1}) of the mantle of octopuses fed frozen crabs or the artificial diet, and of the artificial diet and the crabs used to feed the octopuses

Composition (g kg^{-1})	Octopus fed crab	Octopus fed diet	Frozen crab	Artificial diet
Ash	79 ± 11	134 ± 18	220 ± 14	129 ± 12
Lipids	66 ± 10	46 ± 13	47 ± 15	215 ± 12
Protein (N × 6.25)	740 ± 21	659 ± 32	563 ± 21	421 ± 24
Crude fibre	14 ± 3	15 ± 2	25 ± 5	25 ± 4
Free nitrogen	43 ± 9	60 ± 11	106 ± 17	169 ± 22

Table 5 Amino acid (AA) composition (grams of AA kg^{-1} of protein) of the mantle of *Octopus maya* used in the experiment and fed frozen crabs, of *Octopus vulgaris* juveniles (14 g wet weight), adapted from Villanueva *et al.* (2004), and of the artificial diet used in the experiment

AA (grams of AA kg^{-1} of protein)	*O. maya*	*O. vulgaris*	Artificial diet
Aspartic acid (ASP)	89 ± 5	60 ± 4	93 ± 4
Threonine (THR)	37 ± 2	25 ± 2	28 ± 2
Serine (SER)	41 ± 1	30 ± 2	42 ± 1
Glutamic acid (GLU)	118 ± 5	101 ± 4	121 ± 4
Glycine (GLY)	51 ± 3	34 ± 2	61 ± 3
Alanine (ALA)	47 ± 2	32 ± 2	58 ± 2
Cystine (CYS)	05 ± 1	06 ± 1	08 ± 1
Valine (VAL)	27 ± 1	23 ± 2	27 ± 1
Methionine (MET)	18 ± 1	15 ± 1	23 ± 1
Isoleucine (ISO)	28 ± 1	25 ± 2	15 ± 1
Leucine (LEU)	62 ± 3	44 ± 2	57 ± 2
Tyrosine (TYR)	28 ± 2	25 ± 3	23 ± 2
Phenyalanine (PHE)	29 ± 2	24 ± 2	28 ± 1
Histidine (HIS)	14 ± 1	14 ± 1	17 ± 1
Lysine (LYS)	65 ± 3	44 ± 3	51 ± 2
Tryptophan (TRP)	06 ± 1	–	08 ± 1
Arginine (ARG)	50 ± 2	43 ± 3	45 ± 2
Proline (PRO)	37 ± 1	23 ± 2	49 ± 2
Total AA	766 ± 8	–	768 ± 7

(<0.5% BW per day). FR obtained with any of those diets (<3% BW per day) were low (Castro 1991; Castro *et al.* 1993; Castro & Lee 1994; Domingues 1999; Domingues *et al.* 2005), and one of the reasons for this could be that cuttlefish (*S. officinalis*), used by these authors, do not accept prepared diets as easily as octopuses, e.g. *O. maya*. Results obtained during the past few years and from this experiment are similar to the ones obtained when changing from natural to artificial diets with fish (Dabrowski *et al.* 1978; Lindberg & Doroshov 1986).

GR obtained here with frozen crabs (between 4.3% and 2.2% BW per day) are between the limits reported for cephalopods (Amaratunga 1983; Lee 1994; Lee *et al.* 1998; Domingues *et al.* 2002), and also for *O. maya* in particular (<5% BW per day: Van Heukelem 1983; 4.1% BW per day: Hanlon & Forsythe 1985) for the complete life cycle. This indicates, as expected, that the frozen crabs were a good diet for *O. maya*. During the juvenile phase (the one used in this experiment), GR of 4% BW per day at 20 °C and 7.8% BW per day at 30 °C, decreasing afterwards for preadults and adults, were reported by Van Heukelem (1983) for *O. maya*. A decrease in GR with increasing size is usual in cephalopods, as it is a proportional relation between total weight gained and total animal weight (Forsythe & Van Heukelem 1987; Domingues *et al.* 2002).

No natural mortality was observed on octopuses fed on the artificial diet, which produced no growth during 40 days ($P > 0.05$). This diet seemed to be on the limit of the maintenance level for this species. Animals never stopped eating, contrary to previous experiments using artificial diets to feed *S. officinalis* (Domingues 1999: Domingues *et al.* 2005). The fact that one octopus maintained without food for 50 days had lost 1.5% BW per day (Domingues, unpublished data) could also indicate that the artificial diet has some nutritional value, although not enough to promote growth in *O. maya*. The authors realize nevertheless that just one animal ($n = 1$) being starved is not enough to reach a solid conclusion on weight loss during a period of time. Furthermore, this animal was in an experimental tank and stopped eating after a few days of the experiment, being deprived of food only after this. This could indicate that the animal was sick, or not in good health. Nevertheless, we believe that this weight loss in such a short time, added to the good growth rates for octopuses fed on crabs indicates that the artificial diet had some nutritional value. In fact, the nutritional value that allowed octopuses fed on the artificial diet to maintain their weight could have been supplied by the squid meat used in the artificial diet.

FR with frozen crabs (between 5% and 7% BW per day in WW) were within the ranges for cephalopods, and octopuses in particular, reported in the literature (Choe 1966; Van Heukelem 1976; Amaratunga 1983; Mangold 1983; Forsythe & Van Heukelem 1987; Domingues *et al.* 2001a,2004). These values are normal for sub-adult forms of *O. maya* at this temperature, as animals tend to spend a considerable part of the day hiding in wholes or laying camouflaged on the bottom, requiring less energy compared with more active cephalopods such as cuttlefish or squid. This is supported by the finding that in many occasions, octopuses only consumed one of the frozen crabs (of the two composing the daily ration) leaving the other untouched. On the contrary, the artificial diet was almost always avidly and entirely consumed in both daily feeding periods, which indicates that octopuses were constantly unsatisfied with the energy obtained from the artificial diet. Animals fed on frozen crabs during this experiment produced almost no faeces, and the percentage of faecal remains is probably similar to the ones reported by Van Heukelem (1983).

As the artificial diet contained considerably less water than the frozen crabs, FR on DW were close to the ones for the frozen crab (around 8% BW per day). This diet did not produce weight loss, unlike the majority of artificial diets previously tested in cephalopods (Castro 1991; Castro *et al.* 1993; Castro & Lee 1994; Domingues 1999; Domingues *et al.* 2005). The fact that the diet was consistently accepted during the 40-day experiment (and afterwards, as the animals that were not sacrificed were maintained during 40 more days eating the artificial diet) is an important advance compared with previous research, where after 2 weeks cephalopods reduced consumption considerably (Castro 1991; Castro *et al.* 1993; Castro & Lee 1994; Domingues 1999). This fact, added to the considerable amount of faeces produced when feeding on the artificial diet will allow determination of several factors that can explain the poor performance of the diet, after analysis of the energetic value and biochemical composition of the faeces.

FC (on WW basis) when using frozen crabs fall within the ones reported in the literature for cephalopods (Richard 1971; Pascual 1978; Forsythe *et al.* 1994; Domingues *et al.* 2001a, 2002, 2003, 2004, and for *O. maya* in particular, with conversion rates between 30% and 60% (Hanlon & Forsythe 1985), and of 40% (Van Heukelem 1976,1983).

The energy balance of each diet clearly shows (Table 3) that the artificial diet had high energy content, but was not efficiently assimilated by the octopuses. Because of this, energy in the faces was almost 77%, with less than 5% of the energy lost through faeces when feeding frozen crabs. According to Van Heukelem (1983), energy losses to faeces also represent 5% of the food ingested in *O. maya*. Clearly, the artificial diet ingested provided more energy than the frozen crab. Absorption was clearly more efficient with the frozen crabs (>95%), compared with the artificial diet (<25%). Also, the amount of faeces produced (and collected) when eating the artificial diet was clearly greater, frequently over 35% of the food ingested, while faeces from animals fed on the frozen crabs were scarce, representing less than 5% of the food ingested. These combined factors could explain the absence of growth when eating the artificial diet, despite its high energy content, and consumption rate.

From the analysis in Table 5, which indicates amino acid composition of the mantle of octopuses fed on frozen crabs, we can observe that amino acid composition was similar to the one in the artificial diet. The amino acid composition of octopus mantle was used as a guideline to elaborate further artificial diets tested in subsequent experiments with artificial diets.

One possible reason that could have contributed to the absence of growth with this artificial diet could be its high lipid content (215 g kg^{-1}) compared with the frozen crab, or the lipid mantle content of the octopus (Table 4). Diets with high lipid concentrations promote lower growth in *O. vulgaris*, as lipids are believed to interfere with protein absorption. Also, diets rich in lipids cause damage to the nidamentary glands (Jiménez & García 2002). The high lipid composition of the artificial diet is because of the fact that the diet formulation used a commercial feed that was based on shrimp food with high lipid content, not useful in cephalopods for growth (especially neutral lipids). According to Miliou *et al.* (2005), there is an interaction between temperature, body weight, growth, protein and lipid use. Octopuses prefer crustaceans, and grow larger when fed on crustaceans compared with fish (Nixon 1966; Castro & Guerra 1990; Domingues *et al.* 2003, 2004).

This is the first time that artificial diets were fed to the Yucatan octopus (*O. maya*). Although no growth (or acceptable growth) was obtained, as in previous research with cephalopods using artificial diets, the extremely good acceptance of the artificial diets makes it an interesting cephalopod to continue research on this subject. During future experiments, diets based on the flesh of frozen crabs, with fewer lipids and fibres (that increase the speed at which nutrients pass through the digestive tract, lowering absorption) will be used. Finally, in the elaboration of such a diet, we will try to obtain a similar composition to the one from amino acid composition of the octopuses' mantle analysed during this experiment. Texture and attractiveness of the artificial diet was very acceptable. This might indicate that, for cephalopods, semi-humid diets could be more attractive than dry diets used previously (Castro 1991; Castro *et al.* 1993; Castro & Lee 1994; Domingues 1999), both in attractiveness, and palatability (e.g. texture).

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