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Investigation of the potential effect of diet, body mass and maturity on growth and feed performance of common octopus *Octopus vulgaris*: an information theory approach

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Abstract

The potential effect of body mass (*m*), maturity stage (*ms*), food type (*ft*), food protein (*p*) and lipid (*li*) content, and food protein-to-energy ratio, P/E (*pe*) on Specific Growth Rate (*SGR*, % day^{-1}), Absolute Feeding Rate (*AFR*, g day^{-1}), Feed Efficiency (*FE*, %), Assimilation Efficiency (*AE*, %), and Protein Retention Efficiency (*PRE*, %) in the common octopus was investigated. Six food types were provided *ad libitum*: shrimp, squid, hake, mussel, sardine and artificial one (gels made of hydrated squid flour agglutinated with sodium alginate). Estimated *SGRs*, *AFRs*, *FEs*, *AEs* and *PREs* were modelled with General Linear Models based on an information theory approach, using *m*, *ms*, *ft*, *p*, *li* and *pe* as potential predictor variables. *SGR* decreased when *m* increased; octopuses fed on shrimps showed the highest *SGRs* and the ones fed on mussels showed the lowest *SGRs*. *AFR* increased with *m*. Maximum and minimum *FEs* were observed, when food provided was shrimps and mussels, respectively. Maximum *PRE* was performed by octopuses fed on shrimps or sardines and minimum *PRE* by octopuses fed on mussels. Octopuses fed on artificial diet reached satisfactory levels of *SGR* (0.50% day^{-1}) and *FE* (12.3%).

KEY WORDS: Akaike's Information Criterion, artificial diet, feed performance, growth, Information Theory, *Octopus vulgaris*

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Introduction

The common octopus *Octopus vulgaris* fulfils the expectations of industrial culture, because of its rapid growth (Mangold 1983; Iglesias *et al*. 2000), high fecundity (Mangold 1983), high feed efficiency (Mangold & Von Boletszky 1973; Wells 1978; Mangold 1983), short life cycle (Mangold & Von Boletszky 1973; Katsanevakis & Verriopoulos 2006), easy adaptation to captivity conditions (Iglesias *et al*. 2000), acceptance of non-living, non-motile foods (Boucaud-Camou & Boucher-Rodoni 1983), high nutritional value for human consumption, because of its high n-3 PUFA levels (Sinanoglou & Miniadis-Meimaroglou 1998) and its high market price, combined with increasing global market requirements (Vaz-Pires *et al*. 2004).

Several studies have been carried out, providing information about the nutritional requirements of common octopus *O. vulgaris* and how they affect growth (Lee 1994; García García & Aguado Giménez 2002; Aguado Giménez & García García 2002; García García & Cerezo Valverde 2006; Cerezo Valverde *et al*. 2008; Quintana *et al*. 2008). However, many crucial questions remain unanswered, while in some cases results are controversial.

Octopus vulgaris is characterized by rapid growth, with large individual variability (O'Dor *et al*. 1984; Domain *et al*. 2000). Numerous biotic and abiotic factors are capable of dramatically modifying its growth (Forsythe & Van Heukelem 1987). Individual variability in growth, food intake, feed efficiency and nutritional behaviour of *O. vulgaris* become more intense in industrial culture conditions, where competition for food and territory, cannibalism and reproductive activity are observed (Wells *et al*. 1983; O'Dor *et al*. 1984).

Growth rates and feed efficiencies performed by benthic octopuses fed on artificial diets, such as *O. vulgaris* (Cerezo

Valverde *et al*. 2008; Quintana *et al*. 2008) and *Octopus maya* (Domingues *et al*. 2007; Rosas *et al*. 2007, 2008), were found inferior to those obtained with natural diets. These results indicated that there is a gap in the available knowledge for the successful design of an artificial diet for these organisms.

An artificial diet for octopus should be characterized by specific organoleptic features, which correspond to its feeding mechanisms and the structure of its upper and lower digestive system, i.e.: (1) Stable structure: octopuses often do not consume food right after its dispensing (Petza D., personal observation), so it is vital that it remains stable in the water. (2) Palatability: the chemotactile capacity is much developed in the octopus and involves responses to acids, sugars, amino acids and nucleotides (Wells *et al*. 1965; Chase & Wells 1986). (3) Large size: the octopus has a beak in its buccal cavity, allowing the partition of big food particles before its ingestion (Altman & Nixon 1970). Granulated, extruded and semi-moist feeds, such as those designed for fish, are not acceptable, because they disintegrate during the manipulation by the octopus. (4) Consistency: the octopus does not have the capacity to chew its food, because its buccal cavity lacks a chewing device (i.e. teeth). Right after the partition of food particles by the beak, digestive enzymes are excreted outside the buccal cavity, by glands in the periphery of the arms ring (Nixon 1984) and inside the buccal cavity by the salivary glands. Then food is converted to a fluid pulp, which is finally sucked up by the lips of the buccal cavity. (5) Appropriate composition: artificial food should include all the essential nutrient components (such as amino acids, lipid acids, vitamins, trace elements, etc.) for the specific species, and its composition should meet the special demands of each developmental stage.

In this study, an information theory approach was applied to investigate the potential effects of diet, body mass and maturity stage on growth and feed performance of *O. vulgaris*. For this purpose, rearing experiments were conducted on octopuses of various sizes and various maturity stages, fed on six different food types (five natural and one artificial). The design of an appropriate and effective artificial diet for the octopus and the comparison of its performance with the performance of the natural ones were an additional aim of this study.

Materials and methods

Laboratory animal sampling

Animals used in this study, were hand-collected by SCUBA and free diving in the Saronicos Gulf (37°30′N-37°55′N, 23°E-24°E). Right after their collection, octopuses were immediately placed into 40-L plastic holding tanks, with sufficient aeration provided by portable air pumps (HAILEA, Guangdong Province, China), until their transportation to the laboratory facility, where each octopus was placed individually in 50-, 110- or 190-L holding tanks, depending on its size. All tanks were connected to a 2-m^3 closed seawater system, filled with natural seawater of 38.5 g L^{-1} salinity. The closed seawater system is described in Katsanevakis *et al*. (2005).

To minimize animal stress, a plastic pot was placed in all holding tanks, to be used by the octopuses as a den and the sides of the holding tanks were covered with a black self-adhesive surface. Special attention was also given so that the ratio of holding tank volume to animal body mass was more than 150 L kg^{-1}.

Diets

Six different food types were provided to six groups of laboratory animals, five natural and one artificial. Natural food types were as follows: squid (*Loligo vulgaris*), sardine (*Sardina pilchardus*), hake (*Merluccius merluccius*), mussel (*Mytilus galloprovincialis*) and shrimp (*Parapenaeus longirostris*) and were provided as defrosted, viscera-free fillets. All these food types are readily consumed by the common octopus, and their selection was mostly based on the differentiation of their biochemical composition, i.e. foods with the largest possible variation in their characteristics were included.

Bearing in mind the fact that an appropriate artificial diet for octopus should be characterized by specific organoleptic features (see introduction) and after a long period of experimental trials for the optimization of its characteristics (in terms of composition, structure, behaviour of the diet and its acceptance by the experimental animals), an artificial diet was designed.

Artificial food type design was based on gel formation technology, which consists in enclosing the nutrient compounds in a gel made of natural molecules (i.e. agar, collagen, gelatin, alginates, etc.). Nutrient substances remain enclosed in the grid formed by the gel and consequently they do not diffuse in water. Artificial food provided in this study consisted of hydrated squid flour transformed into gels, with the addition of sodium alginate as agglutination factor.

Squid flour was prepared in the laboratory facilities by freeze-drying squid paste for 72 h in a freeze-drying device (FreeZone 4.5, LABCONCO, Kansas City, MO, USA). Then squid flour was hydrated with distilled water in 1 : 1

ratio of squid flour/distilled water and agglutinated with 20 g kg^{-1} aqueous solution of sodium alginate (FLUKA, Sigma Aldrich, Buchs, Switzerland) in 1 : 0.6 ratio of hydrated squid flour/aqueous solution of sodium alginate. Small spherical proportions (approximately 10 g) of agglutinated squid paste were subjected to a 3-hour coagulation bath, in a 20 g kg^{-1} aqueous solution of calcium chloride (CARLO ERBA REAGENTI, Rodano, Italy), in order for the gels to be formed.

Experimental procedure

Experimental procedure included four feeding periods (Table 1). At the beginning of each feeding period, all animals were subjected to a 30-day acclimatization period, during which they adapted to their new environment and became familiar with the food-dispensing procedure and food type provided. During the acclimatization period, each animal was fed on the same food type, intended to be provided during the experimental period.

The 30-day acclimatization period was followed by a 10-day experimental period. In each experimental period, six laboratory animals participated and each of them was fed on one of the six different food types (the five natural and the one artificial). A total number of twenty four specimens ($n = 24$) were used in the four feeding periods performed (Table 1).

Two different batches of natural food were supplied; the first batch was provided to animals participating in feeding periods 1 and 2, and the second batch to animals participating in feeding periods 3 and 4. Only one batch of artificial food was prepared and provided to all animals throughout the whole experimental procedure.

Because all specimens were collected from the sea, it was impossible to obtain replicates of the same body mass or to form dietary groups (i.e. groups consisted of animals fed on the same food type) with exactly the same body mass range (Miliou *et al.* 2005). Nevertheless, the average initial body mass did not differ significantly among the six dietary groups (ANOVA, $P > 0.05$) and special effort was given in order for the body mass range to be as narrow as possible in all six dietary groups. Furthermore, body mass was included as a covariate in the set of candidate models so that its potential effect would be taken into account.

During the experimental periods, food was provided *ad libitum* once a day precisely at 1300 h. On a daily basis and approximately 1 h after food dispensing, food leftovers were collected and their dry mass was estimated by thermal drying in an oven (U30; MEMMERT GmbH & Co.KG, Schwabach, Germany) at 105 °C for 24 h. Conversion factors of dry-to-wet mass for each food type were estimated and were used to convert the dry mass of food leftovers to wet mass. To calculate the food quantity consumed daily by each octopus, the food leftovers' wet mass was subtracted from the wet mass of the food provided. The estimation of consumed food was done on a dry basis, to avoid the effect of changing moisture content during the 1-hr immersion in

Table 1 Description of the experimental procedure performed for the investigation of the potential effect of diet, body mass and maturity on growth and feed performance of twenty-four common octopus *Octopus vulgaris* specimens, fed on five natural (shrimp, squid, hake, mussel, sardine) and one artificial (gels made of hydrated squid flour agglutinated with sodium alginate) food type

Experimental procedure		Acclimatization period (30 days) + experimental period (10 days)					
Feeding period 1	Animal	1	2	3	4	5	6
	Food type	Shrimp	Squid	Hake	Mussel	Sardine	Artificial
	Food batch	1st	1st	1st	1st	1st	1st
		Acclimatization period (30 days) + experimental period (10 days)					
Feeding period 2	Animal	7	8	9	10	11	12
	Food type	Shrimp	Squid	Hake	Mussel	Sardine	Artificial
	Food batch	1st	1st	1st	1st	1st	1st
		Acclimatization period (30 days) + experimental period (10 days)					
Feeding period 3	Animal	13	14	15	16	17	18
	Food type	Shrimp	Squid	Hake	Mussel	Sardine	Artificial
	Food batch	2nd	2nd	2nd	2nd	2nd	1st
		Acclimatization period (30 days) + experimental period (10 days)					
Feeding period 4	Animal	19	20	21	22	23	24
	Food type	Shrimp	Squid	Hake	Mussel	Sardine	Artificial
	Food batch	2nd	2nd	2nd	2nd	2nd	1st

seawater. The faeces of each octopus were collected every day from the outflow water, using a 500-μm nylon net, attached to the outflows of the holding tanks. Faeces that remained at the bottom or the sides of the tanks were removed by sucking through a tube, which ended to a 500-μm nylon net. The faeces produced by each specimen were preserved at −18 °C; and after the end of each feeding period, their dry mass was estimated. The possibility of underestimating the amount of faeces produced because of bacterial activity and lixiviation should be mentioned.

Throughout the feeding periods, temperature remained constant at 20.0 ± 0.5 °C, salinity at 38.5 ± 0.2 g L^{-1}, while pH ranged between 7.8 and 8.1. There was a photoperiod of 12 h of light/12 of hours darkness, with light period between 0700 and 1900 h. Nitrite and unionized ammonia was $<0.1 \times 10^{-3}$ and $<0.05 \times 10^{-3}$ g L^{-1}, respectively.

All specimens were weighted at the beginning and the end of the experimental period, without been anesthetized, keeping the handling procedure as brief and calm as possible to minimize stress.

When each experimental period ended, animals were immediately euthanized. For this purpose, a mechanical–physical method was used, consisting of immediate placement on an ice-water mix, followed by decapitation. These methods were chosen because they cause the least possible pain and distress and are proposed in experiments, where tissues are harvested for biochemical post-mortem examination (Hellebrekers *et al.* 2001). Each specimen was dissectioned to determine sex and stage of maturity (stages 1 to 6 for each sex), according to Nigmatulin (1977).

Finally, octopuses were homogenized using a tissue homogenizer (A950 food processor attached to KM001 kitchen machine; KENWOOD, Tokyo, Japan), freeze-dried (FreeZone 4.5; LABCONCO, Kansas City, MO, USA) and preserved at −18 °C, until the conduction of the biochemical analyses. The procedure described earlier was also followed for each batch of all food types provided, i.e. squid, shrimp, mussel, hake, sardine and artificial.

Laboratory animal use and well-being

Because the present study involved living animals held in captivity (i.e. laboratory conditions) for a long period of time (approximately 40–45 days), special attention was given for their humane use and well-being. Thus, the principles and recommendations of the Laboratory Animal Science were taken into account, which are recapitulated on its main guideline, the 'Three R concept: Replacement, Reduction, Refinement' (Russell & Burch 1959).

Refinement, which refers to any decrease in the incidence or severity of painful or distressing procedures, was achieved by optimizing the following: (a) collection (e.g. hand-collection), (b) transportation (e.g. direct transportation, use of air-pumps), (c) husbandry (e.g. adequate size of holding tanks and volume of available water, individual rearing, placement of a plastic pot and black cover at the tanks, supply of natural sea water, water recycling, long acclimatization period, regular food dispensing and faeces removal, brief and calm handling during weighting procedure, distress and instantaneous euthanasia) and (d) environmental conditions (e.g. frequent monitoring of temperature, salinity, pH, nitrite and unionized ammonia concentration, application of photoperiod).

Reduction, which refers to a decrease in the number of animals required for a given experiment, was achieved by: (a) choosing and designing the most suitable experimental procedure for the aim of the present study, (b) controlling the environmental factors and (c) applying the most appropriate statistical analysis for the given data, in order for the results to be as informative as possible.

Replacement, which refers to the substitution of living animals by alternative procedures (e.g. *in vitro* techniques, computerized models, etc.), was not applicable in this study.

All these contributed to the humane use and well-being of the laboratory animals, while at the same time a sufficiently informative dataset was collected.

Biochemical analysis

All experimental animals (n = 24) and faeces (n = 24) were subjected to moisture, crude protein and gross energy content determination. Food types (n = 11, two batches of the five natural food types and one batch of the artificial food type) were subjected to moisture, ash, crude protein, crude lipid and gross energy content determination. All the above-mentioned biochemical analyses were performed in triplicate.

As the flesh composition of fish, crustaceans and molluscs varies with season and area of catch (Silva & Chamul 2000), it was considered necessary to estimate the proximate composition for both batches of natural food types provided.

Dry matter was obtained by thermal drying in an oven (U30; MEMMERT GmbH & Co.KG) at 105 °C for 24 h. Ash was determined by incineration in a muffle furnace (ELVEM, Spata, Greece) at 550 °C for 24 h. Crude protein (nitrogen × 6.25) was determined according to the Hach *et al.* (1985) method, and crude lipids were extracted according to the Folch *et al.* (1957) method. Gross energy content was estimated by burning samples in a bomb calorimeter (Calorimetersystem C4000A; IKA® Werke GmbH & Co.KG, Staufen, Germany).

For each food type, the protein-to-energy ratio (P/E) was estimated, using the formula: $P/E =$ [crude protein content of food (in g)] / [gross energy content of the food (in MJ)].

Definition of performance indices

Specific Growth Rate (SGR, % day^{-1}), Absolute Feeding Rate (AFR, g day^{-1}), Feed Efficiency (FE, %), Assimilation Efficiency (AE, %) and Protein Retention Efficiency (PRE, %) were estimated for each specimen for the experimental period, using the following equations, respectively: $SGR = 100\ (\ln m_{fw} - \ln m_{iw})\ t^{-1}$, $AFR = FI_w\ t^{-1}$, $FE = 100\ (m_{fd} - m_{id})\ FI_d^{-1}$, $AE = 100\ (C_d - F_d)\ C_d^{-1}$ and $PRE = 100\ [(P_{fd}\ m_{fd} - P_{id}\ m_{id})\ (P_d\ FI_d)^{-1}]$, where m_f and m_i are the final and initial body mass, respectively (in g), t is the experimental duration (in days), FI is the total food quantity consumed (in g), C is the total energy content of food consumed (in J), F is the unabsorbed energy voided through faeces (in J), P_f and P_i are, respectively, the final and initial crude protein content of octopus (in % dry body mass), and P is the crude protein content of food (in % dry mass). In all the above-mentioned equations the indices '*w*' and '*d*' correspond to wet and dry mass, respectively.

Initial and final tissue protein content were considered to be equal (i.e. $P_{id} = P_{fd}$), based on the results of other studies that have revealed that the protein content of octopus tissue is not affected by the animal body mass (Miliou *et al*. 2005). This assumption was further tested by conducting one-way ANOVA to the experimental data of the present study (protein content of octopus tissue, for octopuses of various sizes, fed on six different diets).

Statistical analysis – model selection

Estimated SGR, AFR, FE, AE and PRE for each specimen were modelled with General Linear Models (GLMs; McCullagh & Nelder 1989), based on an information theory approach (Burham & Anderson 2002).

According to the information theory approach, data analysis is taken to mean the integrated process of *a priori* specification of a set of candidate models (based on the science of the problem), model selection based on the principle of parsimony according to Akaike's information criterion (AIC; Akaike 1973) and the estimation of parameters and their precision. Information theory has been increasingly proposed to be a better and advantageous alternative for model selection than traditional approaches, such as hypothesis testing (Burham & Anderson 2002). Information theory has lately been used for model selection in biological studies, e.g. to model absolute (Katsanevakis 2006) or relative (Katsanevakis *et al*. 2007a; Protopapas *et al*. 2007) growth and aquatic respiration (Katsanevakis *et al*. 2007b).

For each response parameter, a set of fifteen candidate models was built using food type (*ft*) and maturity stage (*ms*) as potential factor predictor variables, and body mass (*m*), food protein content (*p*), food lipid content (*li*) and protein-to-energy ratio (*pe*) as potential continuous predictor variables (Table 2). Strongly correlated variables, such as *m* and *ms,* or *p*, *li* and *pe* were not jointly included in any model. *m* was the mean octopus body mass during the experimental period.

Model selection was based on the small-sample, bias-corrected form of Akaike's Information Criterion (AIC_c) (Akaike 1973; Hurvich & Tsai 1989; Burham & Anderson 2002). The AIC_c differences, $\Delta_i = AIC_{c,i} - AIC_{c,min}$, were computed over all candidate models. To quantify the plau-

Table 2 The candidate models studied for the investigation of the potential effect of body mass (*m*), maturity stage (*ms*), food type (*ft*), food protein (*p*) and lipid (*li*) content and food protein-to-energy ratio (*pe*) on Specific Growth Rate (SGR, % day^{-1}), Absolute Feeding Rate (AFR, g day^{-1}), Feed Efficiency (FE, %), Assimilation Efficiency (AE, %) and Protein Retention Efficiency (PRE, %) in common octopus *Octopus vulgaris* specimens fed on six different food types (shrimp, squid, hake, mussel, sardine and artificial). Y is one of SGR, AFR, FE, AE, PRE; k is the total number of estimated parameters (σ^2 included)

Variables	Model symbolism	k
Body mass, *m*	$Y \sim m$	2
Maturity stage, *ms*	$Y \sim ms$	6
Food type, *ft*	$Y \sim ft$	6
Food protein content, *p*	$Y \sim p$	2
Food lipid content, *li*	$Y \sim li$	2
Protein-to-energy ratio, *pe*	$Y \sim pe$	2
Food type, *ft*, and body mass, *m*	$Y \sim ft + m$	7
Food protein content, *p*, and body mass, *m*	$Y \sim p + m$	3
Food lipid content, *li*, and body mass, *m*	$Y \sim li + m$	3
Protein-to-energy ratio, *pe*, and body mass, *m*	$Y \sim pe + m$	3
Food type, *ft*, and maturity stage, *ms*	$Y \sim ft + ms$	12
Food protein content, *p*, and maturity stage, *ms*	$Y \sim p + ms$	7
Food lipid content, *li*, and maturity stage, *ms*	$Y \sim li + ms$	7
Protein-to-energy ratio, *pe*, and maturity stage, *ms*	$Y \sim pe + ms$	7
–	*null*	1

sibility of each model, given the data and the set of fifteen models, the 'Akaike weight' w_i of each model was calculated, where $w_i = \exp(-0.5\Delta_i)/\sum_{j=1}^{15}\exp(-0.5\Delta_j)$. The 'Akaike weight' is considered as the weight of evidence in favour of model *i* being the actual best model of the available set of models (Akaike 1983; Buckland *et al.* 1997; Burham & Anderson 2002). Models with $\Delta_i > 10$ have essentially no support and might be omitted from further consideration, while models with $\Delta_i < 2$ have substantial support (Burham & Anderson 2002).

Analysis of variance (ANOVA) at 95% confidence level was performed for the comparison of means of (i) octopus tissue protein content, body mass, *SGR*, *FE* and *PRE* for each dietary group and (ii) protein content, lipid content and energy content of the six food types provided. In the cases where statistically significant differences were detected, Tukey's HSD multiple range tests were performed, to determine homogeneous groups of means (Zar 1999). All statistical analyses were carried out, using the Statgraphics Plus 5 (Statistical Graphics Corp., Warrenton, VA, USA) software.

Results

Diets biochemical composition

For the six food types provided, moisture ranged between 645.9 ± 0.8 and 817.6 ± 0.7 g kg^{-1}, ash between 9.2 ± 0.7 and 34.1 ± 0.7 g kg^{-1}, crude protein between 102.3 ± 0.7 and 206.6 ± 3.0 g kg^{-1}, crude lipid between 5.6 ± 0.1 and 140.9 ± 1.3 g kg^{-1}, gross energy between 3.65 ± 0.02 and 10.34 ± 0.09 KJ g^{-1} and P/E between 16.5 ± 0.03 and 40.0 ± 0.12 g MJ^{-1} (Table 3).

The comparison of the biochemical composition of the six food types revealed that there was a statistically significant difference between the means of all the six food types provided, in terms of crude protein ($P < 0.001$), crude lipid ($P < 0.001$) and gross energy content ($P < 0.001$). In all the three above-mentioned cases, the Tukey-HSD test classified the six food types in six homogeneous groups, i.e. all food types had significantly different levels of protein, lipid and energy content.

Growth and Feed Performance

The maximum value of *SGR* was 3.02 ± 0.53% day^{-1} (mean value ± standard error) performed by octopuses fed on shrimp and the minimum −0.25 ± 0.27 by ones fed on mussels. The maximum value of *AFR* was 16.10 ± 4.81 g day^{-1} performed by octopuses fed on shrimp and the minimum 5.65 ± 1.82 by ones fed on mussel. The maximum value of *FE* was 48.61 ± 2.34% performed by octopuses fed on shrimp and the minimum 0.15 ± 5.94 by ones fed on mussel. The maximum value of *AE* was 98.96 ± 0.28% performed by octopuses fed on squid and the minimum 96.77 ± 1.03 by ones fed on sardine. The maximum value of *PRE* was 47.42 ± 6.95% performed by octopuses fed on sardine and the minimum 0.71 ± 8.04 by ones fed on mussel (Table 4).

Table 3 Tissue biochemical composition (g kg^{-1} wet mass), gross energy (kJ g^{-1} wet mass) and protein-to-energy ratio, *P/E* (g protein MJ^{-1}) of the natural (two batches of shrimp, squid, hake, mussel, sardine) and artificial (one batch of gels made of hydrated squid flour agglutinated with sodium alginate) foods provided (mean ± standard error)

	Moisture (g kg^{-1})	Ash (g kg^{-1})	Crude protein (g kg^{-1})	Crude lipid (g kg^{-1})	Gross energy (kJ g^{-1})	P/E (g MJ^{-1})
Shrimp						
Batch 1	759.5 ± 3.6	11.4 ± 0.9	192.3 ± 0.8	9.8 ± 0.9	5.42 ± 0.01	35.5 ± 0.15
Batch 2	746.8 ± 1.7	9.9 ± 0.8	206.6 ± 3.0	10.9 ± 1.3	5.75 ± 0.01	35.9 ± 0.52
Squid						
Batch 1	798.4 ± 3.5	21.5 ± 0.3	157.8 ± 2.0	11.3 ± 0.8	4.62 ± 0.03	34.2 ± 0.44
Batch 2	742.2 ± 2.1	17.7 ± 0.1	188.0 ± 0.2	27.8 ± 0.9	6.26 ± 0.03	30.1 ± 0.03
Hake						
Batch 1	801.6 ± 0.4	9.2 ± 0.7	181.3 ± 0.5	5.6 ± 0.1	4.54 ± 0.05	40.0 ± 0.12
Batch 2	781.6 ± 3.9	13.6 ± 0.9	187.1 ± 0.2	11.3 ± 0.1	4.97 ± 0.08	37.7 ± 0.08
Mussel						
Batch 1	817.6 ± 0.7	34.1 ± 0.7	102.3 ± 0.7	11.6 ± 0.2	3.65 ± 0.02	28.0 ± 0.19
Batch 2	788.6 ± 5.9	27.1 ± 0.7	116.8 ± 2.3	17.0 ± 0.4	4.26 ± 0.05	27.5 ± 0.53
Sardine						
Batch 1	680.4 ± 7.1	22.8 ± 0.9	203.9 ± 3.4	78.0 ± 0.7	8.13 ± 0.05	25.1 ± 0.42
Batch 2	645.9 ± 0.8	17.5 ± 0.9	170.3 ± 0.3	140.9 ± 1.3	10.34 ± 0.09	16.5 ± 0.03
Artificial	797.0 ± 1.6	18.0 ± 0.4	151.9 ± 1.2	14.2 ± 0.1	4.59 ± 0.09	33.1 ± 0.27

Table 4 Mean values (± standard errors) of initial wet body mass (m_i), Specific Growth Rate (*SGR*), Absolute Feeding Rate (*AFR*), Feed Efficiency (*FE*), Assimilation Efficiency (*AE*) and Protein Retention Efficiency (*PRE*) for each common octopus *Octopus vulgaris* dietary group (i.e. groups consisted of four octopuses fed on the same diet) performed during the four feeding periods

	Dietary groups					
	Shrimp diet	Squid diet	Hake diet	Mussel diet	Sardine diet	Artificial diet
m_i (g)						
Mean value	411	404	525	214	451	597
Standard error	±222	±91	±139	±56	±109	±176
SGR (% day^{-1})						
Mean value	3.02	0.91	1.72	-0.25	1.34	0.50
Standard error	±0.53	±0.32	±0.75	±0.27	±0.36	±0.55
AFR (g day^{-1})						
Mean value	16.10	12.52	14.72	5.65	12.59	12.32
Standard error	±4.81	±2.74	±3.59	±1.82	±5.12	±2.75
FE (%)						
Mean value	48.61	24.05	42.09	0.15	33.40	12.32
Standard error	±2.34	±3.94	±10.68	±5.94	±4.79	±0.26
AE (%)						
Mean value	98.45	98.96	97.14	98.81	96.77	98.69
Standard error	±0.40	±0.28	±1.31	±0.38	±1.03	±0.51
PRE (%)						
Mean value	46.28	25.05	35.32	0.71	47.42	12.65
Standard error	±2.29	±4.00	±8.11	±8.04	±6.95	±0.13

The assumption of constant protein content of octopus tissue (i.e. $P_{id} = P_{fd}$) that was made for the estimation of *PRE* was tested and was not violated (ANOVA, $P > 0.05$).

General Linear Models and Model Selection

Results of modelling *SGR*, *AFR*, *FE*, *AE* and *PRE* performed by common octopus specimens ($n = 24$) fed on six food types (five natural and one artificial) with General Linear Models and the application of the Akaike Information Criterion for model selection are given in Table 5.

The best model for *SGR* was the one with body mass (m) and food type (ft) as predictor variables, with no other model having substantial support by the data (Table 5). *SGR* decreased when body mass increased and the predictive equations for each food type were as follows: $SGR = a - 1.77\ \log(m)$, where a was 7.42 for shrimp, 6.48 for hake, 6.01 for sardine, 5.75 for squid, 5.32 for artificial diet and 3.77 for mussel (Fig. 1). Three homogeneous groups were revealed by the comparison of *SGRs* with the Tukey-HSD test (Table 6). Octopuses fed on shrimp showed the highest *SGR*, while octopuses fed on mussel showed the lowest *SGR* (Fig. 1).

The best model for *AFR* was the one with body mass (m) as a single predictor variable, with no other model having substantial support by the data (Table 5). *AFR* increased when body mass increased, and the predictive equation was $AFR = -17.9 + 11.7\ \log(m)$ (Fig. 2).

The best model for *FE* was the one with food type (ft) as a single predictor variable, with no other model having substantial support by the data (Table 5). Three homogeneous groups were revealed by the comparison of *FEs* with the Tukey-HSD test (Table 6). Maximum and minimum *FEs* were observed, when food provided was shrimp and mussel, respectively (Fig. 3). Hake and sardine were diets that octopuses had utilized in a sufficient level and the relevant *FEs* did not differ from those of octopuses fed on shrimp. Squid in the form of natural food showed statistically no significant difference with artificial food in terms of induced *FEs*.

In the case of *AE*, four models had substantial support by the data: (i) the one with food lipid content (li) as predictor variable with predictive equation: $AE = 99.4–1.49\ \log(li)$, (ii) the one with body mass (m) as predictor variable with predictive equation: $AE = 102–1.49\ \log(m)$, (iii) the one with food lipid content (li) and body mass (m) as predictor variables with predictive equation: $AE = 102–1.27\ \log(li)–1.23\ \log(m)$ and (iv) the null model (Table 5). Because the null model had substantial support by the data, secure conclusions cannot be drawn for *AE*, based on the present dataset.

The best model for *PRE* was the one with food type (ft) as predictor variable with no other model having substantial support by the data (Table 5). Three homogeneous groups were revealed by the comparison of *PREs* with the Tukey-HSD test (Table 6). Maximum *PRE* was performed by octopuses fed on shrimp or sardine and minimum *PRE* by octopuses fed on mussel (Fig. 4). *PREs* performed by octopuses fed on squid in the form of natural and artificial diet showed statistically no significant difference (Table 6).

In the case of *SGR*, *AFR*, *FE* and *PRE*, the null model did not have substantial support by the data (i.e. $\Delta_i > 2$, Table 5), indicating that the present data set was sufficient to develop

Table 5 Values of residual sum of squares (*RSS*), small-sample bias-corrected form of Akaike's Information Criterion (AIC_c), AIC_c differences (Δ_i) and Akaike's weights (w_i) for each of the candidate models for Specific Growth Rate (*SGR*), Absolute Feeding Rate (*AFR*), Feed Efficiency (*FE*), Assimilation Efficiency (*AE*) and Protein Retention Efficiency (*PRE*) performed by common octopus *Octopus vulgaris* specimens fed on six different food types (shrimp, squid, hake, mussel, sardine and artificial) during the four feeding periods. For each parameter, the best model or models selected by the Akaike's Information Criterion are highlighted with bold characters. In every case, sample size was $n = 24$

Model	*RSS*	AIC_c	Δ_i	w_i	Model	*RSS*	AIC_c	Δ_i	w_i
SGR ~ m	38.5	18.5	13.6	0.1%	***AE ~ m***	**54.1**	**26.7**	**0.7**	**18.2%**
SGR ~ ms	17.3	13.1	8.1	1.5%	*AE ~ ms*	41.8	34.3	8.4	0.4%
SGR ~ ft	14.7	9.2	4.2	10.4%	*AE ~ ft*	40.9	33.8	7.8	0.5%
SGR ~ p	33.2	15.0	10.0	0.6%	*AE ~ p*	58.5	28.6	2.6	7.1%
SGR ~ li	37.6	18.0	13.0	0.1%	***AE ~ li***	**53.4**	**26.4**	**0.4**	**21.3%**
SGR ~ pe	36.7	17.4	12.5	0.2%	*AE ~ pe*	58.2	28.5	2.5	7.5%
SGR ~ ft+m	**10.2**	**5.0**	**0.0**	**85.7%**	*AE ~ ft+m*	39.1	37.3	11.3	0.1%
SGR ~ p+m	31.1	16.3	11.4	0.3%	*AE ~ p+m*	54.1	29.6	3.6	4.3%
SGR ~ li+m	36.9	20.4	15.5	0.0%	***AE ~ li+m***	**50.5**	**27.9**	**2.0**	**9.8%**
SGR ~ pe+m	35.2	19.3	14.3	0.1%	*AE ~ pe+m*	53.8	29.5	3.5	4.5%
SGR ~ ft+ms	7.6	34.8	29.8	0.0%	*AE ~ ft+ms*	27.3	65.5	39.5	0.0%
SGR ~ p+ms	15.7	15.4	10.4	0.5%	*AE ~ p+ms*	38.7	37.1	11.1	0.1%
SGR ~ li+ms	17.3	17.7	12.7	0.1%	*AE ~ li+ms*	41.6	38.8	12.8	0.0%
SGR ~ pe+ms	17.0	17.3	12.4	0.2%	*AE ~ pe+ms*	40.6	38.2	12.2	0.1%
SGR Null	39.7	16.7	11.7	0.2%	***AE* Null**	**58.5**	**26.0**	**0.0**	**26.2%**
AFR ~ m	**951**	**96**	**0.0**	**44.4%**	*PRE ~ m*	8,127	142	17.9	0.0%
AFR ~ ms	983	110	14.6	0.0%	*PRE ~ ms*	6,525	151	27.1	0.0%
AFR ~ ft	968	110	14.2	0.0%	***PRE ~ ft***	**2,011**	**124**	**0.0**	**91.6%**
AFR ~ p	1,184	101	5.3	3.2%	*PRE ~ p*	8,134	142	17.9	0.0%
AFR ~ li	1,223	102	6.0	2.2%	*PRE ~ li*	8,041	142	17.7	0.0%
AFR ~ pe	1,217	101	5.9	2.3%	*PRE ~ pe*	8,224	143	18.2	0.0%
AFR ~ ft+m	802	110	14.3	0.0%	*PRE ~ ft+m*	2,008	129	4.8	8.3%
AFR ~ p+m	931	98	2.4	13.5%	*PRE ~ p+m*	8,023	145	20.6	0.0%
AFR ~ li+m	925	98	2.2	14.5%	*PRE ~ li+m*	7,965	145	20.4	0.0%
AFR ~ pe+m	940	98	2.6	12.0%	*PRE ~ pe+m*	8,101	145	20.8	0.0%
AFR ~ ft+ms	712	144	48.2	0.0%	*PRE ~ ft+ms*	1,862	168	43.2	0.0%
AFR ~ p+ms	949	114	18.3	0.0%	*PRE ~ p+ms*	6,503	156	31.8	0.0%
AFR ~ li+ms	983	115	19.2	0.0%	*PRE ~ li+ms*	6,051	155	30.2	0.0%
AFR ~ pe+ms	979	115	19.1	0.0%	*PRE ~ pe+ms*	6,379	156	31.4	0.0%
AFR Null	1,227	99	3.5	7.8%	*PRE* Null	8,255	140	15.6	0.0%
FE ~ m	8,024	142	16.6	0.0%	*FE ~ li+m*	7,597	144	18.3	0.0%
FE ~ ms	6,180	150	24.7	0.0%	*FE ~ pe+m*	7,187	142	17.0	0.0%
FE ~ ft	**2,107**	**125**	**0.0**	**90.3%**	*FE ~ ft+ms*	1,837	167	41.8	0.0%
FE ~ p	6,357	137	11.2	0.3%	*FE ~ p+ms*	4,952	150	24.5	0.0%
FE ~ li	7,736	141	15.7	0.0%	*FE ~ li+ms*	6,026	154	29.0	0.0%
FE ~ pe	7,272	140	14.3	0.1%	*FE ~ pe+ms*	5,697	153	27.7	0.0%
FE ~ ft+m	2,087	130	4.6	9.1%	*FE* Null	8,089	139	14.1	0.1%
FE ~ p+m	6,332	139	14.1	0.1%					

m, body mass, *ms*, maturity stage, *ft*, food type, *p*, food protein content, *li*, food lipid content, *pe*, protein-to-energy ratio.

predictor models. Maturity stage (*ms*), food protein content (*p*) and protein-to-energy ratio (*pe*) were not included in any of the selected models for the five parameters studied (Table 5).

Discussion

Growth and feed performance

Somatic growth and feed performance are strongly affected by octopus diet, a finding supported by this study (Figs 1, 3 & 4) and also by other relevant studies (García García & Aguado Giménez 2002; Aguado Giménez & García García 2002; Miliou *et al.* 2005; García García & Cerezo Valverde 2006). Diet digestibility and nutrient assimilation ultimately determine the nutritional value of a particular food type; a diet rich in nutrients is not practical if the nutrients cannot be adequately assimilated and utilized, in favour of somatic growth (Lee 1994).

Differences in growth rate and feed efficiency have often been ascribed to different P/E values of the food type pro-

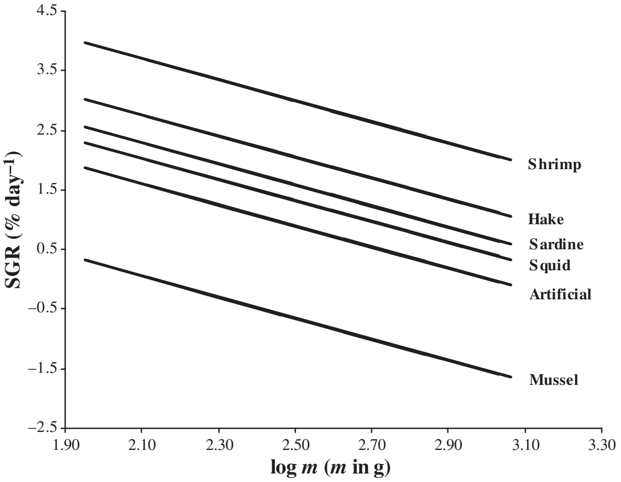


Figure 1 Specific growth rate (SGR) in relation to body mass (m) and food type (ft), based on the best model [$SGR \sim m + ft$] for the common octopus *Octopus vulgaris* fed on six food types (shrimp, squid, hake, mussel, sardine and artificial). Predictive equations for each food type are as follows: $SGR = a - 1.77 \log(m)$, where a is 7.42 for shrimp, 6.48 for hake, 6.01 for sardine, 5.75 for squid, 5.32 for artificial diet and 3.77 for mussel (adj. $R^2 = 65\%$, $P < 0.001$, $n = 24$). Estimated *SGRs* were modelled with General Linear Models (GLMs) and model selection was based on Akaike's Information Criterion (AIC).

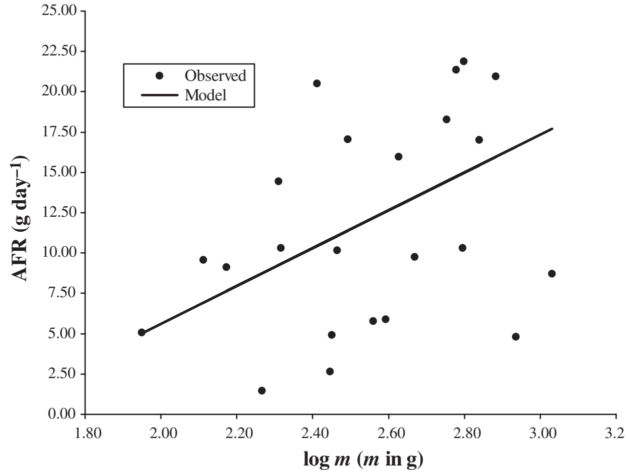


Figure 2 Absolute Feeding Rate (AFR) in relation to body mass (m), based on the best model [$AFR \sim m$] for the common octopus *Octopus vulgaris* fed on six different food types (shrimp, squid, hake, mussel, sardine and artificial). Predictive equation is $AFR = -17.9 + 11.7 \log(m)$ (adj. $R^2 = 19\%$, $P = 0.019$, $n = 24$). Estimated *AFRs* were modelled with General Linear Models (GLMs) and model selection was based on Akaike's Information Criterion (AIC).

vided (García García & Aguado Giménez 2002; Aguado Giménez & García García 2002; Miliou *et al.* 2005; García García & Cerezo Valverde 2006). This is a justification that is not supported by the results of this study, because P/E was a variable that was not selected as predictor in any of the five parameters examined (SGR, AFR, FE, AE and PRE). O'Dor *et al.* (1984) claimed that the optimum P/E for *O. vulgaris* food is around 35, as its natural diet is composed mainly of crab, adding that foods with lower P/E values (e.g. fish) are not energetically efficient.

However, Lee (1994) argued that P/E might not be accurate for the establishment of cephalopod energetic requirements. Alternatively, he suggested that a better way might be to ascertain the food amino acid balance. García García & Cerezo Valverde (2006) found that as bogue proportion was increased in mixed diets with crab, feed efficiency and protein production performed by octopuses were also increased, and they suggested that these results express a more suitable amino acid balance in bogue than in crab. Until today, cephalopod requirements in proteins and amino acids (in terms of quality and quantity) have not yet been determined. Nevertheless, Villanueva *et al.* (2004) and Domingues *et al.* (2007), who determined the mantle amino acid composition of *O. vulgaris* and *O. maya* juveniles, respectively, believe that an effective diet for these species should have amino acid composition similar to the one of the octopuses' mantle.

Table 6 The results of multiple comparisons of SGR, FE and PRE mean values (for each common octopus *Octopus vulgaris* dietary group participated in the four experimental trials) with the Tukey's HSD test. The homogeneous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences at 95% confidence level. (For each dietary group $n = 4$ octopuses)

	Homogeneous groups								
Dietary group	*SGR*			*FE*			*PRE*		
Mussel	X			X			X		
Artificial	X	X		X	X		X	X	
Squid	X	X		X	X	X	X	X	X
Sardine		X	X		X	X			X
Hake		X	X			X		X	X
Shrimp			X			X			X

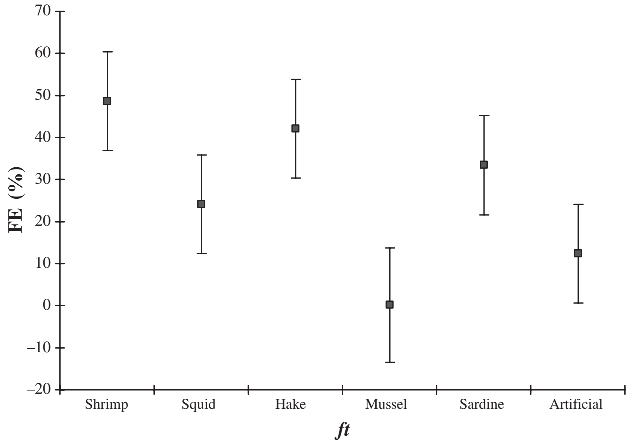


Figure 3 Mean values of Feed Efficiency (*FE*) and 95% confidence intervals for each food type (*ft*), based on the best model [$FE \sim ft$] for the common octopus *Octopus vulgaris* fed on six different food types (shrimp, squid, hake, mussel, sardine and artificial). (adj. $R^2 = 66\%$, $P < 0.001$, $n = 24$). Estimated *FEs* were modelled with General Linear Models (GLMs) and model selection was based on Akaike's Information Criterion (AIC).

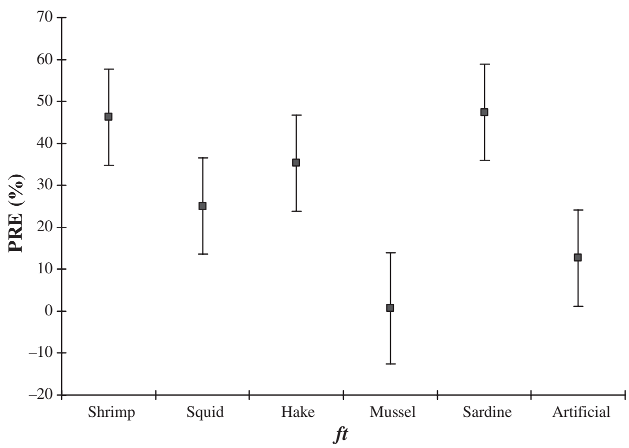


Figure 4 Mean values of Protein Retention Efficiency (*PRE*) and 95% confidence intervals for each food type (*ft*), based on the best model [$PRE \sim ft$] for the common octopus *Octopus vulgaris* fed on six different food types (shrimp, squid, hake, mussel, sardine and artificial). (adj. $R^2 = 69\%$, $P < 0.001$, $n = 24$). Estimated *PREs* were modelled with General Linear Models (GLMs) and model selection was based on Akaike's Information Criterion (AIC).

Several authors have attributed the low growth rates observed by octopuses fed on high-lipid diets, on their limited capacity to metabolize lipids (García García & Aguado Giménez 2002; Aguado Giménez & García García 2002; García García & Cerezo Valverde 2006; Petza *et al.* 2006). By this study, it is revealed that sardine, the richest food type in crude lipid content, caused satisfactory levels of growth rate and feed efficiency, which were also comparable to the other food types provided (except of mussel). Similar levels of those parameters have been reported for the other food types in other studies. This particular case showed that probably the assimilation and utilization of food lipids seems to be a matter of quality rather than quantity. This finding should be specially highlighted because it leads to selective paths of diet in the octopus culture.

Ghiretti & Violante (1964) associated octopus growth with the trace elements content of food consumed, while Castro *et al.* (1993) suggested that high mortality in cuttlefish may be because of copper deficiency. Copper is an essential trace element for the synthesis of haemocyanin, the pigment responsible for the carriage of oxygen in the blood of cephalopods and crustaceans. Castro *et al.* (1993) have observed that dietary restriction causes significant decrease in haemolymph copper levels. This finding supports that cephalopods need to maintain a certain level of copper in their haemolymph and that crustaceans or other species of cephalopods are ideal sources of this element, indicating their necessity in cephalopod diet. Although trace elements requirements of octopus have not yet been identified, there are indications that strontium (Hanlon *et al.* 1989), calcium and sulphur (Villanueva & Bustamante 2006) are included in the requirements at least of certain developmental stages.

According to the relevant literature, growth and feed performance was also improved when octopuses were fed on mixed diets instead of monodiets (Smale & Buchan 1981; García García & Cerezo Valverde 2006).

Taking all these under consideration, it seems likely that octopus growth depends on many nutritional parameters, and it is difficult to be interpreted using only one parameter, such as protein-to-energy ratio or food lipid content.

The negative correlation of growth rates with body mass, found in the present study (Fig. 1), is a result also indicated by other studies (Forsythe & Van Heukelem 1987; García García & Aguado Giménez 2002; Aguado Giménez & García García 2002; Miliou *et al.* 2005). It is a fact that smaller octopuses obtain higher mass standardized metabolic rates in relation to bigger ones, trying to satisfy their energetic needs for growth and development (Forsythe & Van Heukelem 1987; Katsanevakis *et al.* 2005).

García García & Aguado Giménez (2002) and Miliou *et al.* (2005) have also found significant positive correlation between *AFR* and body mass (Fig. 2) in octopuses of the same species, fed on fish (bogue or sardine) and squid, respectively, at 20 °C.

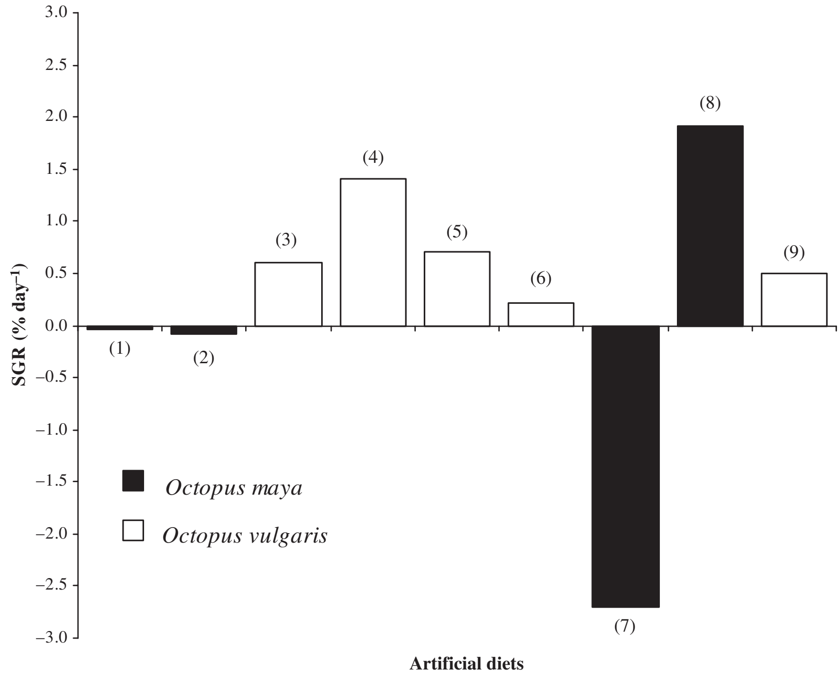
High Protein Retention Efficiency observed in octopuses fed on shrimp and fish (sardine and hake) (Fig. 4), interpreted to high growth rates, because food proteins remain in octopuses tissues and transformed through metabolism to structural and functional proteins, in favour of body mass growth (Bendiksen *et al.* 2003). This is a finding that comes in accordance with other studies results, conducted on various animals (Waterlow 1984; Houlihan *et al.* 1988). *Octopus vulgaris* obtain high growth rates through elevated protein synthesis, which is followed by extremely low protein degradation rate and high retention efficiency of the proteins synthesized (Houlihan *et al.* 1990). The high efficiency of retention of synthesized proteins is probably related to high feed efficiency values in favour of somatic growth (O'Dor & Wells 1987).

Besides *P*/*E*, maturity stage and food protein content were predictor variables that were not selected in any of the models for the five parameters examined (*SGR*, *AFR*, *FE*, *AE* and *PRE*), although: (1) values of crude protein content of the six food types provided were significantly different and (2) all maturity stages (except for the 6th, which is the stage after spawning) were represented in the studied sample. These variables have either no effect in the investigated biological system or small effect that would necessitate a larger dataset to be detected.

Performance of artificial diet

One of the goals of this research was the design of an artificial diet and the study of its performance in comparison with natural ones. A profitable and supportable long-term industrial culture of common octopus depends on the development of a satisfactory artificial diet, which might increase the profitability and minimize the high economical risk (García García *et al.* 2004).

Growth rates performed by octopuses fed on various artificial diets are given in Fig. 5. Domingues *et al.* (2007) studied the effect of a dry pelleted diet on growth and survival of the Yucatan octopus, *O. maya,* and found that the artificial diet did not promote growth and animal did not loose weight, although feeding rates were high. Rosas *et al.* (2007) estimated the effect of an artificial diet on the energy balance of *O. maya* subadult octopuses and found higher production efficiency in octopuses fed on crabs than



(1): Shrimp commercial feed and squid paste (Domingues *et al.*, 2007)
(2): Shrimp pellet and squid paste (Rosas *et al.*, 2007)
(3): Shrimp paste agglutinated with gelatin (Quintana *et al.*, 2008)
(4): Squid paste agglutinated with gelatin (Quintana *et al.*, 2008)
(5): Shrimp and fish paste agglutinated with alginate (Cerezo Valverde *et al.*, 2008)
(6): Shrimp and fish paste agglutinated with gelatin (Cerezo Valverde *et al.*, 2008)
(7): Crab paste agglutinated with alginate (Rosas *et al.*, 2008)
(8): Crab paste agglutinated with gelatin (Rosas *et al.*, 2008)
(9): Hydrated squid flour agglutinated with alginate (Present study)

Figure 5 Specific Growth Rates (*SGR*, % day^{-1}) performed by octopuses fed on various artificial diets.

those fed on artificial diet. Quintana *et al.* (2008) studied the acceptance and the effect on growth of two artificial diets in *O. vulgaris* sub adults. Both diets were acceptable by the animals, and it was found that artificial diet with squid paste performed better results in terms of animals' growth and food conversion efficiency, than those performed by octopuses fed on artificial food made of shrimp paste. Cerezo Valverde *et al.* (2008) conducted a comparable study for the effect of two formulated moist diets i.e. fish and shrimp paste agglutinated either with alginate or gelatin on growth, feed efficiency and condition of *O. vulgaris*. They found that artificial diet with alginate had better performance than the one with gelatin. They highlighted the greater stability and the overall better performance of artificial diet containing alginate as agglutination factor in comparison with gelatin, assuming that poor results of artificial food with gelatin possibly performed because of nutritional value reduction, caused by heat applied in order for the gelatin to be diluted in water. Rosas *et al.* (2008) examined the effect of binder on growth and digestibility in *O. maya* juveniles and concluded that the type of binder affect octopuses growth and survival. Animals fed on crab paste bound with alginate lost weight and died. The authors argued that alginates limit nutrient absorption from the diet and inhibit growth.

As concluded by Rosas *et al.* (2007), cephalopod growth rates, when fed on artificial diets, have produced poor results compared to natural (live or frozen) diets (Castro 1991; Hanlon *et al.* 1991; Lee *et al.* 1991; Castro *et al.* 1993; Castro & Lee 1994), indicating that the information available to adequately formulate diets for these organisms is limited. However, the present study, as well as the results of Cerezo Valverde *et al.* (2008), revealed the good performance of alginates as binders. Alginates as agglutination factors are used in the food industry, because of their colloidal properties and their capacity to form gels (Hoek van den *et al.* 1995). The fact that alginates are marine products (because they derive from brown algae cell wall) amplifies their use in artificial diets for marine species.

Octopuses fully accepted the artificial diet provided in the present study, which was based on squid flour. It is worth-mentioning that the performance of the artificial diet gave statistically similar results when compared to the performance of frozen squid. This finding along with Miliou *et al.* (2006) and Quintana *et al.* (2008) study results imply that squid seems to be a sufficient diet for the common octopus.

As it is mentioned earlier (see Introduction), an artificial diet for octopus should be characterized by specific features, many of which are fulfilled by using squid as a raw material. Quintana *et al.* (2008) refer that squid artificial diets are characterized by better consistency (in relation to artificial diets based on other raw materials e.g. shrimp). It is suggested that this could be associated with squid's better cohesion because of a higher percentage of collagen in its tissue (Ando *et al.* 2001). As far as squid's biochemical composition and its nutritional value concerns, it is noteworthy that the fatty acid composition of squid-fed octopuses matches the ideal i.e. 'natural' fatty acid profile of *O. vulgaris* (Miliou *et al.* 2006). This means that when octopuses are fed on a monodiet consisted only of squids under captivity conditions, their fatty acid profile does not change significantly in relation to the wild one's, who have vast nutritional choices. Moreover, squid diet fulfils the condition of DHA/EPA ration of approximately 1.5, which according to Okumura *et al.* (2005) is necessary for the normal growth and development of the common octopus paralarvae. Indeed, Miliou *et al.* (2006) found that squid-fed octopuses showed a DHA/EPA ratio more that 1.5.

The novelty of the artificial food, designed in this study, was that its preparation was based on dry raw material (squid flour), a feature that encloses many advantages over the wet ones e.g. direct availability, repeatable and stable composition, effortless maintenance and transportation, minimal microbiological charge, least manipulation, lowest preparation time, etc. The use of gels formation technology in artificial food production may offer better results in the future, because it provides the capability to include in the gels components such as vitamins, fatty acids, trace elements, attractants, taste-enhancing substances, etc. This will presumably improve the performance of artificial diets in terms of palatability, digestibility, assimilation or energy benefit and also reduce production cost, in favour of octopus industrial culture.

Acknowledgements

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