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Effects of food thermal treatment on growth, absorption, and assimilation efficiency of juvenile cuttlefish, *Sepia officinalis*

Pedro M. Domingues · Lorenzo Marquez · Nelda López · Carlos Rosas

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Abstract The diet of frozen grass shrimp (*P. varians*) was compared to similar grass shrimp that had suffered either boiling, drying at 60°C, or freeze-drying by lyophilization at −40°C. In experiment 1, cuttlefish fed the frozen shrimp were significantly larger ($P < 0.05$) at the end of 10 days and at the end of the experiment, compared with those fed the boiled or dried shrimp. Growth rates were also higher for cuttlefish fed the frozen shrimp, compared with the remaining two. Growth rates were also higher for cuttlefish fed the frozen shrimp, compared with the remaining two. In experiment 2, there were no differences in weight ($P > 0.05$) between cuttlefish fed the frozen or the freeze-dried shrimp, whereas cuttlefish fed the dried shrimp were smaller at the end of the experiment. Growth rates of cuttlefish fed the dried shrimp were lower, compared with those for cuttlefish fed the frozen and freeze-dried shrimp, with no significant differences ($P > 0.05$) between them. Cuttlefish fed freeze-dried and frozen shrimp showed a higher trypsin activity compared to animals fed boiled and dry (60°C) shrimp. A higher proportion of absorbed energy was channelled into biomass production in animals fed frozen and freeze-dried shrimp (56% and 43%, respectively) than for animals fed oven-dried (60°C) or boiled shrimp. The heat treatment suffered by the shrimp, either dry or wet, negatively affected diet quality, probably due to denaturation, and loss (by boiling) of proteins and amino acids. Additionally, the heating processes may have oxidized the lipids to a large extent, contributing to the loss of the polar lipids (polyunsaturated fatty acids), which are essential for cephalopods as for other organisms. Freeze-drying by lyophilization (negative temperatures) did not affect the nutritional quality of the shrimp.

Keywords Cuttlefish · Diets · Heat treatment · Lyophilization · Nutrition

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Introduction

S. officinalis has been cultured in the laboratory around the world, from the early 1960s until the present day (Schroder 1966; Richard 1966, 1971, 1975; Pascual 1978; Yim 1978; Boletzky 1979, 1983; Boletzky and Hanlon 1983; DeRusha et al. 1989; Forsythe et al. 1994; Lee et al. 1998; Domingues 1999; Bettencourt 2000; Domingues et al. 2001b; 2002, 2004, 2006; Sykes et al. 2006). Cuttlefish have to be fed live prey during the first 2-3 weeks of their life (Richard 1975; Forsythe et al. 1994, Domingues 1999, Domingues et al. 2001a, 2004). Usually, mysid shrimp are used as first prey. After this period, either as juveniles (De Rusha et al. 1989; Forsythe et al. 1991; Domingues et al. 2001b; Koueta and Boucaud-Camou 1999; 2001; Koueta et al. 2002) or adults (Richard 1971; Pascual 1978; Domingues et al. 2002, 2004; Sykes et al. 2006), this species accepts dead food such as frozen shrimp, crabs or fish.

The two main factors that have prevented large-scale culture are the dependence on live prey during the first part of the life cycle (O'Dor and Wells 1987; Lee et al. 1991; Lee 1994; Domingues 1999) and the lack of a successful artificial diet for cephalopod culture (Domingues et al. 2007a). Considerable cost reductions of up to 80% can be estimated, particularly when using commercial production methods, if prepared diets could successfully replace natural prey (Domingues et al. 2005, 2006).

The development of artificial diets for cephalopods is a recent research field, having started in the early 1990s (Castro 1991; Lee et al. 1991; Domingues 1999; Domingues et al. 2005). Early feeding experiments using *S. officinalis* were done with either moist or dry pellets (Castro 1990; Lee et al. 1991; Castro et al. 1993) or surimi (fish myofibrillar protein concentrate; Castro et al. 1993; Castro and Lee 1994; Domingues 1999; Domingues et al. 2005). These experiments demonstrated that large juveniles and adults readily accepted prepared diets. Feeding rates for *S. officinalis* on prepared diets were always considerably lower than a normal laboratory maintenance diet of crustaceans (Pascual 1978; Richard 1971, 1975; Boletzky 1979; Castro et al. 1993; Castro and Lee 1994; Lee et al. 1991; Forsythe et al. 1994; Domingues et al. 2001b, 2002, 2003a, b, 2004, 2006, 2007a; Koueta and Boucaud-Camou 1999, 2001; Koueta et al. 2000). Nevertheless, high feeding rates on prepared diets were reported for the Yucatan octopus, *Octopus maya* (Domingues et al. 2007b; Rosas et al. 2007). Growth and survival of cephalopods with these diets are comparable to fish larvae being transitioned from natural to prepared diets (Dabrowski et al. 1978; Lindberg and Doroshov 1986). Consequently, the formulation of such a diet is still in the early stages, being one priority for the successful large-scale culture of cephalopods (Lee 1994; Domingues et al. 2006, 2007a, b).

Despite moderate acceptance of prepared diets, negative growth with artificial diets was common, with the highest growth rates reported in the literature being close to 0.5% BW day^{-1} (Castro 1990; Castro et al. 1993; Castro and Lee 1994; Lee et al. 1991; Domingues 1999; Domingues et al. 2005). The reported growth rates are ten times lower than those with natural diets, composed mainly of crustaceans (Pascual 1978; Lee et al. 1991; Forsythe et al. 1994; Domingues et al. 2001b, 2002; Sykes et al. 2003). Furthermore, mortality is usually higher compared with natural diets (DeRusha et al. 1989; Lee et al. 1991; Castro et al. 1993).

Besides low palatability (and consequent low feeding rates), semimoist or dry diets used for cephalopod research are mainly composed of fish feeds (or other marine organisms), and usually suffer thermal treatments during preparation. Therefore, we designed two experiments to determine the effects of heating and/or loss of water in the quality and performance of grass shrimp (*Paleomonetes varians*) as the only food for cuttlefish. This

prey was used to assure that cuttlefish would have high feeding rates (contrary to artificial diets) since they resemble in shape the live or dead shrimp even after thermal treatment.

Material and methods

The natural diet of frozen grass shrimp (*P. varians*) was compared to similar grass shrimp that had suffered either one of the following three thermal treatments: (1) boiling at 100°C for 1 min, (2) drying in an oven at 60°C during 16 h, or (3) freeze drying by lyophilization at −40°C during 16 h. Data collected from both experiments were used to calculate: (1) the mean instantaneous growth rate (MIGR); (2) the feeding rate (FR) (% body weight day^{-1}) = (FI/average $W(t)$) × 100, where FI is the food ingested and average $W(t)$ is the average wet weight of the cuttlefish during the time period (t); and (3) food conversion (FC) = (W2 − W1)/FI, where W2 − W1 is the weight gained by the cuttlefish during the time period. Cumulative mortality was accounted for in all the diets tested.

Organisms and culture system

Juvenile *S. officinalis* used in this experiment were born from eggs laid in the laboratory by females cultured in the Centro IFAPA Agua del Pino, Cartaya, Spain. The culture system for both experiments was the same, being composed of 18 rectangular white trays with bottom area of 2,840 cm^2 and average water depth of 8 cm (approximately 40 L of seawater) connected to a flow-through system. During experiment 1, water temperature varied between 20.5 ± 1°C, while in experiment 2, temperature varied between 21.5 ± 1°C. Salinity was 37 ± 1 ppt and dissolved oxygen 95 ± 1%, with a water flow of 20 l h^{-1}, for both experiments. Each tank had an independent water inlet, outlet, and aeration, and was covered with a white plastic mesh so that cuttlefish could not jump out. Low light intensity was used to maintain low stress levels (Koueta and Boucaud-Camou 2003). Lights were only turned on during feeding, faeces collection, and cleaning periods. Cuttlefish were fed frozen shrimp (*Palaemonetes* sp.) until the start of each experiment during the acclimation period. This period lasted for 1 week.

At the end of experiment 1, ten animals that had been fed the frozen shrimp, and six animals fed either boiled and dried shrimp diets were sacrificed. Also, at the end of experiment 2, 12 animals (two from each replicate) from each of the three diets tested were also sacrificed. Samples of the mantle tissues and digestive gland were collected for posterior analysis. For every animal sacrificed in both experiments, body weight and digestive gland weight were recorded in order to establish the digestive gland weight/body weight relation.

Experiment 1

The natural diet of frozen grass shrimp (*P. varians*) that served as the control was compared with similar grass shrimp that had suffered the following two thermal treatments: (1) boiling at 100°C for 1 min, or (2) drying in an oven at 60°C during 16 h. At the start of the experiment, 4-month-old juveniles were randomly placed in the experimental trays. A total of 90 juvenile *S. officinalis* were used. Five cuttlefish were randomly placed in each of the 18 trays used. Initial cuttlefish weight in all 18 replicates was similar ($P > 0.05$). An initial mean wet weight of 12.5 ± 1.0 g, 12.6 ± 0.6 g, and 12.4 ± 0.6 g, was recorded for the

animals in the six replicates fed the frozen, dried, and boiled shrimp, respectively. There were no differences between them ($P > 0.05$), when grouping all the weights of the 30 cuttlefish fed each of the three diets.

Feeding rate of cuttlefish was 12% BW day^{-1}, which is an adequate feeding rate for this species at these culture temperatures (DeRusha et al. 1989; Koueta and Boucaud-Camou 1999; Domingues et al. 2002; 2003a, 2004). Food was provided once a day, at 09:00 h. The food remained in the culture trays between approximately 5 and 6 h, and then uneaten remains were removed and weighed. In the case of the dried shrimp, uneaten remains were dried for 16 h in the oven and then weighed. Each tank had at least three shrimps per animal, to assure all cuttlefish had food available. Faeces were collected from each tank on a daily basis (14:00 h), before removing the food remains from the trays. Faeces from each diet were pooled. The energy from each of the three diets and from faeces was measured directly from caloric value measured in a calorimetric pump (Parr$^{@}$). Cuttlefish were all weighed individually, every 10 days. The experiment lasted for 20 days, as at the end of this period differences in growth were already marked, and digestive glands were large enough to allow determination of the enzymatic activity and further biochemical composition studies.

Experiment 2

The natural diet of frozen grass shrimp (*P. varians*) that served as the control was compared to similar grass shrimp that had suffered the following two thermal treatments: (1) drying in an oven at 60°C during 16 h, and (2) freeze drying by lyophilization at −40°C during 16 h. Younger cuttlefish (3-month-old juveniles) were used than in experiment 1, since they were the ones available at the moment. A total of 72 juvenile *S. officinalis* were used, and randomly placed in the experimental trays. Four cuttlefish were randomly placed in each of the 18 trays used. Initial cuttlefish weight in all 18 replicates was similar ($P > 0.05$). An initial mean wet weight of 5.45 ± 0.56 g, 5.88 ± 0.27 g, and 6.00 ± 0.35 g, was recorded for the animals in the six replicates fed the frozen (control), dried (60°C) and freeze dried shrimp, respectively. There were no differences between them ($P > 0.05$), when grouping all the weights of the 24 cuttlefish fed each of the three diets.

Feeding rate of cuttlefish was 12% BW day^{-1}, and food was provided once a day, at 09:00 h. Again, food remained in the culture trays approximately between 5 and 6 h, and then uneaten remains were removed and weighed. In the case of the dried and the freeze-dried shrimp, uneaten remains were dried for 16 h in the oven and then weighed. Each tank had at least three shrimps per animal, to assure all cuttlefish had food available. Faeces were collected from each tank on a daily basis (14:00 h), before removing the food remains from the trays. Faeces from each diet were pooled. The energy from each of the three diets and from faeces was measured directly from caloric value measured in a calorimetric pump (Parr$^{@}$). Cuttlefish were all weighed individually, every 10 days. The experiment lasted for 40 days, to assure that animals had sufficient digestive gland and caecum that would be used for determinations of digestive enzymes activity and posterior biochemical analysis.

Partial energy balance

Total ingested energy was calculated as $I = IR \times EFC$, where IR is the ingestion rate g day^{-1} g^{-1} of animal and EFC is the energy food content (J g^{-1}) (Lucas 1993). The

absorbed food was obtained by calculation of the absorbed efficiency (AbEf, %) defined by Condrey (1972) as $AE = [(I' - F')/(1 - F')I'] \times 100$, where I′ is the ratio of the ash free dry weight (AFDW) to the dry weight (DW) of the food and F′ is the ratio of the AFDW to DW of the faeces. The DW and AFDW of food and faeces were obtained by placing food and faeces samples at 60°C to dry until constant weight and by placing samples in a muffle furnace at 500°C for 4 h, respectively. The energy absorbed was calculated as: $Ab = I \times AE$. Energy produced (P) was calculated using the actual growth rate of the cuttlefish obtained during experimental time. The value of 18.8 ± 0.72 J g^{-1} DW was used to transform the growth data into production units (P; J g^{-1} DW day^{-1}). This value was obtained from analyzing energy content applied to ten whole animals by means of a calorimeter (Parr®), previously calibrated with benzoic acid.

Enzymatic activity

Enzymatic activity was measured individually in animals fed during 20 and 40 days, during experiments 1 and 2, respectively. Digestive glands were dissected, freeze-dried, and stored until analysis. All the animals were fasted for 12 h before sampling. Samples for total proteases and trypsin were homogenized at 4°C in Tris-base buffer (Tris base 0.09 M, Boric acid 0.08 M, EDTA 2 mM, mercapto-ethanol 0.5 M, glycerol 10%, pH 8.3). Samples for acid phosphatases were homogenized after an osmotic shock (KCl 1%, EDTA 1 mM) to provoke release of the intracellular content (Perrin et al. 2004). Homogenates were centrifuged at 13,200 rpm for 20 min at 4°C. The supernatant was diluted in ten volumes of the extraction buffer.

The soluble-protein content was measured in diluted homogenates (Bradford 1976) using the Bio-Rad protein determination kit (Biorad®-500-0006). Samples were read in a Biorad model 550 microplates reader at 495 nm. Assays were made in triplicate for each sample. Acid proteases activity was measured in homogenates using yellow casein (0.0005%) in phosphate buffer (KH_2PO_4 0.096 M, NaH_2PO_4, 0.004 M, pH 3). Digestive gland was homogenized and incubated during 1 h at 37°C; absorbance was read at 442 nm optical density (OD). One unit was defined as the amount of enzyme that catalyzes the release of dye causing a $\Delta A/\Delta t = 1$ OD min^{-1}. Assays were made in triplicate for each sample. Trypsin activity was measured in diluted (1:100) homogenates using Na-benzoyl-L-arginin-p-nitro-anilide (BAPNAl 1 mM) as a substrate in a buffer (0.1 M TRIS, pH 8). Samples were incubated at 25°C for 1 h. Absorbance was read at 410 nm. Acid phosphatases were measured in homogenates using p-nitrophenil-phosphate (2%) in a buffer Tris–HCl (1 M). Samples were incubated during 30 min at 25°C and stopped with addition of 1 ml of NaOH 1 M (Perrin et al. 2004).

Statistics

After each weighing period, differences in weight between the three groups were determined by means of statistical analysis, using the program STATISTICA 6.0. Analyses of variances (ANOVAs) and Tukey's tests (when differences were found between treatments) were performed between the three replicates of each group (Zar 1999), and if no significant differences were found between the three replicates, all cuttlefish in those groups fed the same diet were gathered, and an ANOVA between the three diets (Zar 1999) was performed. ANOVAs were also performed to compare growth, feeding rates, food conversions, energetic balance, and enzyme activities between diets.

Results

Experiment 1

Table 1 shows results for growth (g), growth rates, feeding rates (% BW day^{-1}), and food conversions (%) of cuttlefish fed the three diets. Cuttlefish fed the frozen shrimp were significantly larger ($P < 0.05$) at the end of the experiment, compared to the ones fed the boiled or dried shrimp. Also, they were larger ($P < 0.05$) at the end of every weighing interval, when comparing with the previous one. Cuttlefish fed the boiled shrimp did not grow ($P > 0.05$), and cuttlefish fed the dried shrimp were larger than the ones fed the boiled shrimp at the end of the experiment ($P < 0.05$), as well as when compared with their initial weight. Growth rates for the entire experiment were also higher ($P < 0.05$) for cuttlefish fed the frozen shrimp (2.0 ± 0.6% BW day^{-1}) compared with the remaining two. Similarly, overall food conversions for cuttlefish fed the frozen shrimp (23.1 ± 5.1%) were higher ($P < 0.01$) compared to those obtained for animals fed the boiled (3.0 ± 4.5%) and dried shrimp (1.4 ± 6.5%), which were not significantly different ($P > 0.05$) from each other. Average feeding rates on the three diets were not different

Table 1 Growth of juvenile cuttlefish in weight (g), growth and feeding rates (% BW day^{-1}) and food conversions (%) fed frozen shrimp, dried shrimp (in an oven at 60°C during 16 h) and boiled shrimp (at 100°C during one minute) during experiment 1, which lasted for 20 days

Diet type	Weight		
	d 1 (g)	d 10 (g)	d 20 (g)
Frozen shrimp	12.5 ± 1.0 a	16.2 ± 1.0 a	18.8 ± 1.4 a
Dried shrimp	12.6 ± 0.6 a	13.0 ± 0.5 b	14.2 ± 1.1 b
Boiled shrimp	12.4 ± 0.6 a	12.4 ± 0.9 b	12.4 ± 1.4 c
		GR%	
		1–10 d	10–20 d
Frozen shrimp		2.6 ± 0.6 a	1.5 ± 0.4 a
Dried shrimp		0.3 ± 0.6 b	0.6 ± 0.3 b
Boiled shrimp		0.0 ± 0.6 b	−0.3 ± 0.5 c
		FC%	
		1–10 d	10–20 d
Frozen shrimp		33.5 ± 5.3 a	20.9 ± 4.9 a
Dried shrimp		0.8 ± 5.9 b	5.1 ± 3.7 b
Boiled shrimp		0.7 ± 7.4 b	−3.8 ± 5.4 c
		FR%	
		1–10 d	10–20 d
Frozen shrimp		7.7 ± 0.6 a	7.0 ± 1.0 a
Dried shrimp		8.5 ± 0.5 a	8.4 ± 0.7 a
Boiled shrimp		7.7 ± 0.6 a	7.7 ± 1.0 a

The different letters indicate significant differences between the three diets

($P > 0.05$) in any weighing interval, nor for the total length of the experiment, and varied between 7.5% and 8.4% BW day^{-1} for the three diets, being slightly higher ($P > 0.05$) for cuttlefish fed the dried shrimp.

The ratio (%) between digestive gland and body weight was 5.5 ± 1.0 for cuttlefish fed the frozen shrimp (control), compared with 3.9 ± 0.9 for cuttlefish fed the boiled shrimp, and 4.0 ± 0.7 for cuttlefish fed the dried shrimp. The percentage of the digestive gland in weight, in relation to total body weight was significantly higher ($P < 0.05$) for animals fed the frozen shrimp, compared with the ones fed the boiled and dried shrimp; these last two were not statistically significantly different ($P > 0.05$). There was a positive correlation ($r = 0.94$) between digestive gland and body weight (Fig. 1). Separate correlations between digestive gland and body weight for animals fed each diet also showed strong correlation ($r > 0.80$) for all three diets tested.

No animals fed either the frozen or the dried shrimp died during the experiment. Nevertheless, three cuttlefish fed the boiled shrimp diet in one replicate between days 1–10 of the experiment, and therefore this replicate was eliminated. Another cuttlefish fed this diet died between days 10 and 20; in this case, it was identified as the smallest and removed from the replicate. Results for day 10–20 from this replicate were still included in the results of the experiment by counting only four animals in this tank during this period.

Partial energetic balance showed that cuttlefish fed frozen shrimp channelled 66% more energy to biomass production than animals fed dry shrimp as a consequence of a higher absorption efficiency (AbEf,%; fed frozen shrimp 89%, fed dry 48%, and fed boiled shrimp 55%) (Table 2). In fact a value of zero energy channelled to biomass production despite the ingested energy was similar between treatments (Table 2).

Experiment 2

There was no mortality for cuttlefish fed the frozen shrimp during the experiment. Two animals fed the freeze-dried shrimp died, one in one replicate between days 10 and 20, and another in a different replicate between days 20 and 30. Nevertheless, both were identified, and therefore these replicates were accounted for, both with three animals each for the next

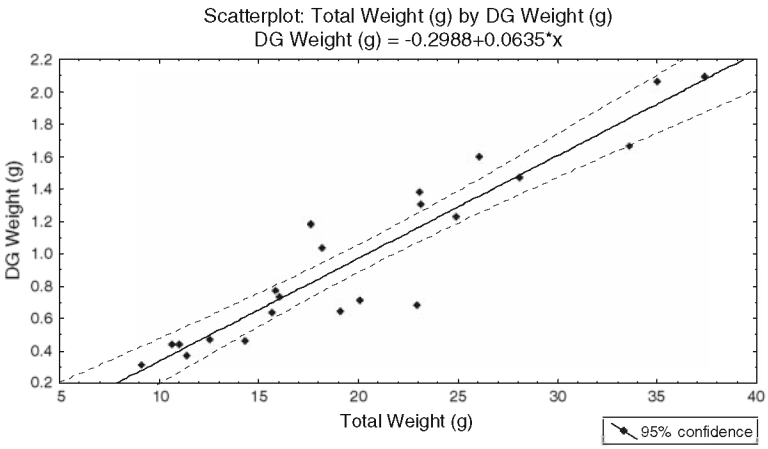

Fig. 1 Relation between digestive gland weight and total body weight for cuttlefish fed three diets (frozen, dried, and boiled shrimp) during 20 days in experiment 1 ($r = 0.94$)

Table 2 Partial energetic balance of *S. officinalis* fed frozen shrimp, dry shrimp (60°C), boiled shrimp (experiment 1) and frozen shrimp, dry shrimp (60°C) and freeze dry shrimp (experiment 2). Values are means ± standard errors (SEs)

Exp. 1		Exp. 2	
Ingested energy (I) (Joules/day/g WW)			
Type of food			
Frozen	443.0 ± 35.4 b	Frozen	544.3 ± 47.352 b
Dry (60°C)	457.1 ± 36.6 b	Freeze dry	581.9 ± 50.625 b
Boiled	381.4 ± 30.5 a	Dry (60°C)	473.4 ± 41.185 a
Absorbed energy (Ab) (Joules/day/g WW)			
Type of food			
Frozen	392.8 ± 31.5 b	Frozen	482.6 ± 39.453 b
Dry (60°C)	218.3 ± 33.9 a	Freeze dry	521.9 ± 44.396 b
Boiled	211.0 ± 36.6 a	Dry (60°C)	226.1 ± 47.064 a
Faeces (Joules/day/g WW)			
Type of food			
Frozen	50.2 ± 4.1143 a	Frozen	61.6 ± 5.0548 a
Dry (60°C)	238.7 ± 19.575 c	Freeze dry	60.0 ± 4.9192 a
Boiled	170.4 ± 13.977 b	Dry (60°C)	247.2 ± 20.274 b
AbEf (%)			
Type of food			
Frozen	88.7 ± 5.802 b	Frozen	88.7 ± 5.802 b
Dry (60°C)	47.8 ± 3.1256 a	Freeze dry	89.7 ± 5.8685 b
Boiled	55.3 ± 3.6191 a	Dry (60°C)	47.8 ± 3.1256 a
Production (P) (Joules/day/g WW)			
Type of food			
Frozen	315.0 ± 35.375 c	Frozen	270.8 ± 30.413 b
Dry (60°C)	105.9 ± 11.894 b	Freeze dry	225.2 ± 25.291 b
Boiled	0.0 ± 0 a	Dry (60°C)	94.5 ± 10.614 a
P/I × 100			
Type of food			
Frozen	71.1 ± 17.3 c	Frozen	49.8 ± 16.7 b
Dry (60°C)	23.2 ± 7.9 b	Freeze dry	38.7 ± 14.4 b
Boiled	0.0 ± 0.9 a	Dry (60°C)	20.0 ± 10.5 a
P/Ab × 100			
Type of food			
Frozen	80.2 ± 16.5 c	Frozen	56.1 ± 19.7 b
Dry (60°C)	48.5 ± 10.1 b	Freeze dry	43.2 ± 18.8 b
Boiled	0.0 ± 0.7 a	Dry (60°C)	41.8 ± 10.5 a

Different letters indicate statistical differences at $P < 0.05$

weighing periods. Similarly, two cuttlefish fed the dried shrimp died during the experiment. In this case, both casualties were in the same replicate between days 10 and 20. Therefore, this replicate was eliminated, and this diet remained with five replicates for the rest of the experiment.

Growth of cuttlefish fed the three diets is shown in Fig. 2. During all weighing intervals, there were no differences in weight ($P > 0.05$) between cuttlefish fed the frozen or the freeze-dried shrimp. Final living weight was of 12.86 ± 1.48 g and 11.52 ± 1.27 g, for cuttlefish fed frozen and freeze-dried shrimp, respectively. In contrast, at every weighing interval and at the end of the experiment, cuttlefish fed the dried shrimp (7.36 ± 0.82 g) were smaller ($P < 0.05$) compared with the ones fed the other two diets.

Figure 3 shows the growth rates for cuttlefish fed the three diets. During every weighing interval, and for the complete experiment, growth rates of cuttlefish fed the dried shrimp were lower ($P < 0.05$), compared with cuttlefish fed the frozen and freeze-dried shrimp. When comparing growth rates for cuttlefish fed the frozen and freeze-dried shrimp, there were no differences ($P > 0.05$) at every weighing interval. Overall growth rates for the experiment were similar ($P > 0.05$) for cuttlefish fed frozen shrimp (2.1 ± 0.4% BW day^{-1}) and freeze-dried shrimp (1.8 ± 0.4% BW day^{-1}). Average growth rate for cuttlefish fed dried shrimp for the entire experiment was 0.5 ± 0.2% BW day^{-1}.

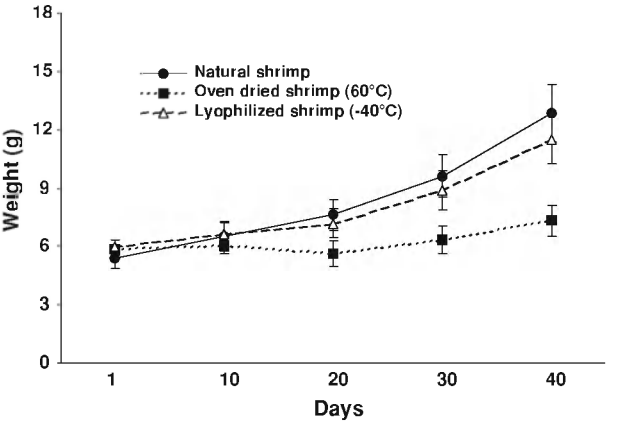

Fig. 2 Growth in wet weight (g) of juvenile cuttlefish fed three diets (frozen, dried or freeze-dried shrimp) during 40 days. Bars indicate standard deviations

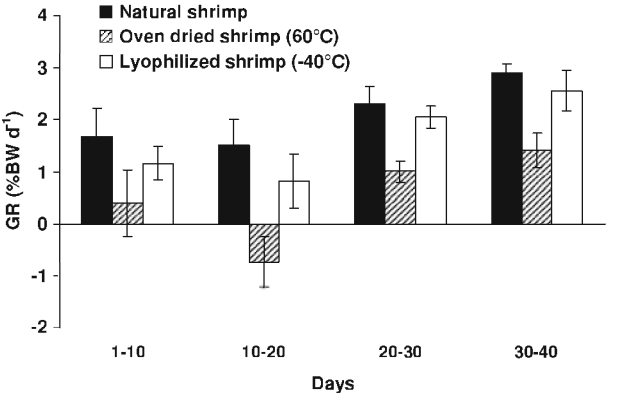

Fig. 3 Growth rates (% body weight/day) of juvenile cuttlefish fed three diets (frozen, dried or freeze-dried shrimp) during 40 days. Bars indicate standard deviations

Feeding rates for the experiment are shown in Fig. 4. There were no differences in feeding rates ($P > 0.05$) for the three diets tested. Average feeding rates for the experiment were of 8.6 ± 0.7% BW day^{-1}, 8.7 ± 0.6% BW day^{-1}, and 8.7 ± 0.8% BW day^{-1} for cuttlefish fed the frozen, hydrolyzed, and dried shrimp, respectively.

Food conversions for the experiment are shown in Fig. 5. During the first half of the experiment, food conversions were always different ($P < 0.05$) between cuttlefish fed the three diets, with the ones fed the frozen diet converting better than the ones fed the freeze-dried shrimp, which also converted better compared with those fed the dried shrimp. During the second half of the experiment, cuttlefish fed the frozen and freeze-dried shrimp had similar ($P > 0.05$) food conversions; both were higher than with the dried shrimp. Average food conversions for the experiment were of 25.7 ± 4.2%, 20.4 ± 4.8%, and 6.6 ± 7.9% for cuttlefish fed the frozen, freeze-dried, and dried shrimp, respectively.

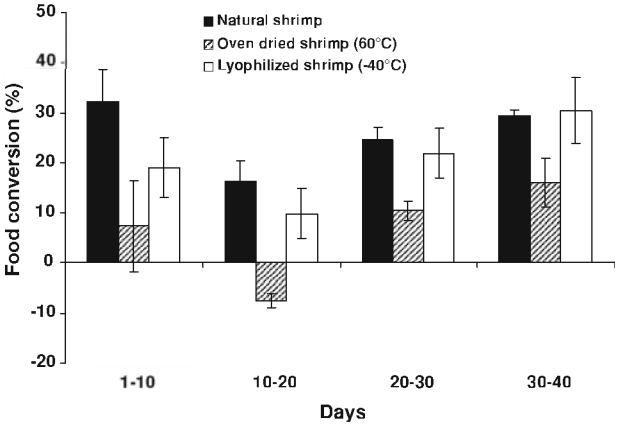

Fig. 4 Food conversions (%) of juvenile cuttlefish fed three diets (frozen, dried or freeze-dried shrimp) during 40 days. Bars indicate standard deviations

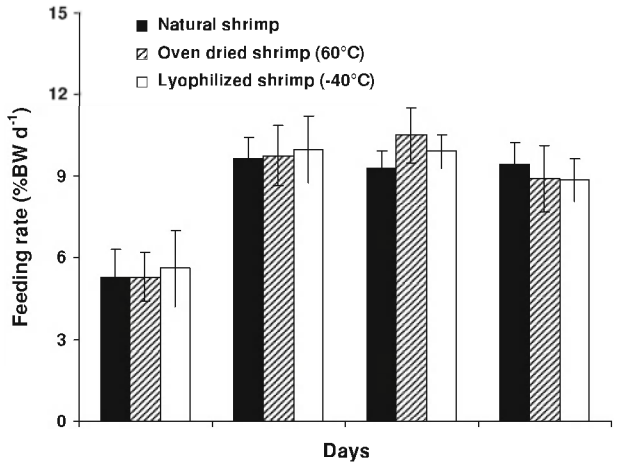

Fig. 5 Feeding rates (% body weight/day) of juvenile cuttlefish fed three diets (frozen, dried or freeze-dried shrimp) during 40 days. Bars indicate standard deviations

There were no differences ($P > 0.05$) between cuttlefish fed the frozen and the freeze-dried shrimp, but both groups had better conversion ($P < 0.05$) than those fed dried shrimp.

The ratio (%) between digestive gland and body weight was of 5.4 ± 0.8 for cuttlefish fed the frozen shrimp (control), compared with 4.9 ± 1.0 for cuttlefish fed the freeze-dried shrimp, and 3.7 ± 0.3 for cuttlefish fed the dried shrimp. The percentage of the digestive gland in weight in relation to total body weight was not different ($P > 0.05$) for animals fed the frozen and freeze-dried shrimp, but both were higher compared with the ones fed the dried shrimp. There was a positive correlation ($r = 0.90$) between digestive gland and body weight (Fig. 6). Similarly to experiment 1, separate correlations between digestive gland and body weight for animals fed each diet also showed strong correlations ($r > 0.80$) for all three diets tested.

Partial energetic balance showed that cuttlefish fed frozen and freeze-dried shrimp channelled a higher proportion of ingested and absorbed energy than animals fed oven-dried (60°C) shrimp ($P < 0.05$; Table 2). AbEf (%) was two times higher in animals fed frozen and freeze-dried shrimp (89%) than cuttlefish fed the dried shrimp (48%). In consequence a higher proportion of absorbed energy was channelled to biomass production in animals fed frozen and freeze-dried shrimp (56% and 43%, respectively) than in animals fed oven-dried (60°C) shrimp (Table 2).

Enzyme activity

Higher enzyme activity of acid phosphatases and acid proteases was observed in digestive glands of animals fed boiled shrimp than in animals fed the dried and frozen shrimp (experiment 1, Fig. 7), with values significantly different ($P < 0.05$) between the three diets. The enzyme activities of acid phosphatases and acid proteases were similar ($P > 0.05$) for cuttlefish fed freeze-dried or frozen shrimp, and both presented higher activity compared with cuttlefish fed the dried shrimp (experiment 2, Fig. 7). It is interesting to note that the difference between enzyme activity of animals fed dry (60°C) shrimp and boiled shrimp (experiment 1) was greater than the difference observed with animals fed freeze-dried or frozen shrimp in experiment 2 (Fig. 7).

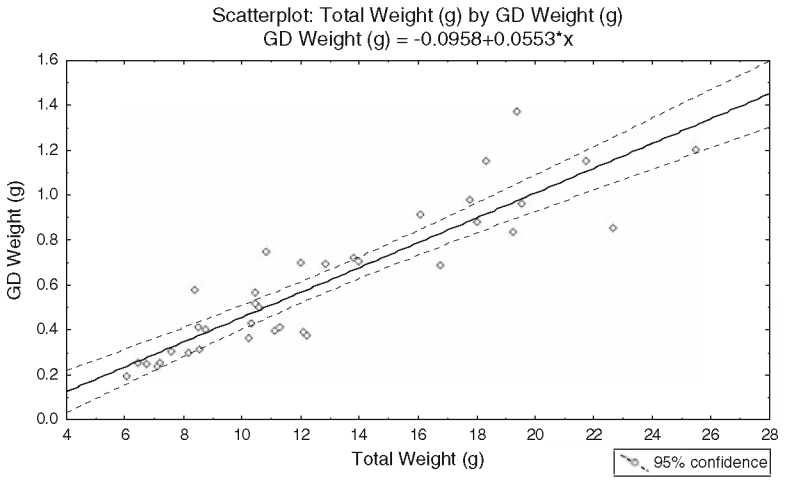

Fig. 6 Relation between digestive gland weight and total body weight for cuttlefish fed three diets (frozen, dried, and freeze-dried shrimp) during 40 days in experiment 2 ($r = 0.90$)

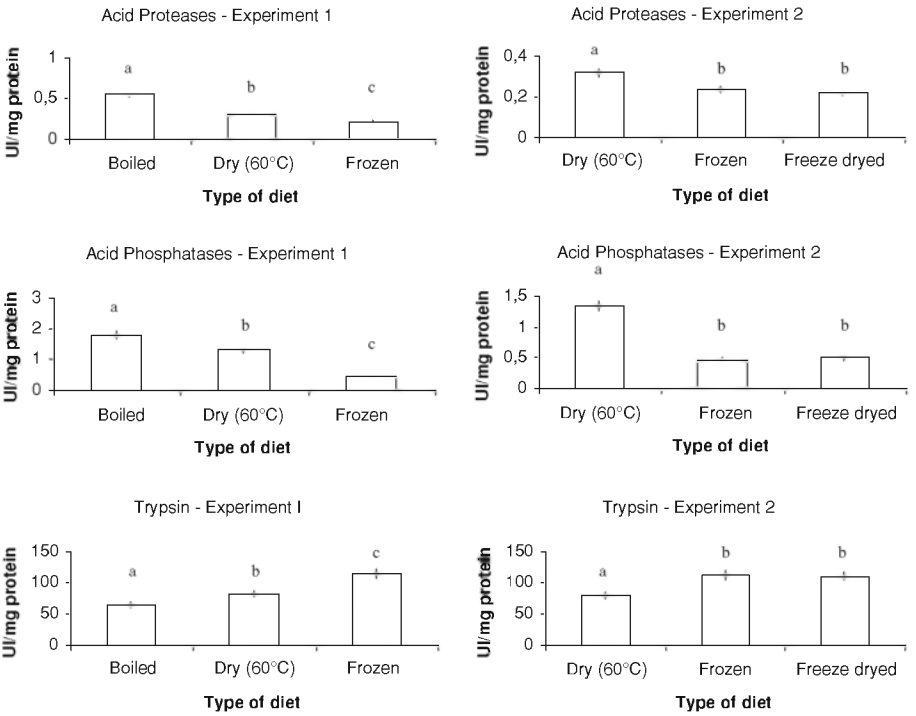

Fig. 7 Enzyme activity in digestive gland of cuttlefish fed frozen, dried, and boiled shrimp (experiment 1), and frozen, dried, and freeze-dried shrimp (experiment 2). Bars indicate standard deviations and letters indicate significant differences ($P < 0.05$)

In contrast, an inverse trypsin activity was observed; animals fed boiled shrimp had lower activity ($P < 0.05$) than those fed dried shrimp, and these also had lower activity ($P < 0.05$) than the ones fed frozen shrimp (experiment 1, Fig. 7). Trypsin activity was similar ($P > 0.05$) for cuttlefish fed freeze-dried and frozen shrimp, and both had higher ($P < 0.05$) trypsin activity than animals fed dried (60°C) shrimp (experiment 2, Fig. 7).

The comparison between enzyme activity of acid phosphatases, acid proteases, and trypsin with the diets that were used in both experiments (frozen and dried at 60°C shrimp) can provide information on how age (cuttlefish of 4 months old in experiment 1 and 3 months old in experiment 2) affects enzyme activity. Results obtained indicate that age did not affect enzyme activity of animals fed frozen and dried shrimp. All comparisons between enzyme activity between animals of different ages, fed either frozen or dried shrimp, were not significantly different ($P > 0.05$). Acid proteases activity was 0.30 ± 0.05 μg/mg and 0.32 ± 0.06 μg/mg of protein, when fed dry shrimp and 0.22 ± 0.03 μg/mg and 0.24 ± 0.04 μg/mg of protein when fed frozen shrimp, for 4- and 3-month-old cuttlefish, respectively. Similarly, acid phosphatases activity was 1.30 ± 0.15 μg/mg and 1.32 ± 0.12 μg/mg of protein, when fed dry shrimp, and 0.45 ± 0.08 μg/mg and 0.47 ± 0.07 μg/mg of protein, when fed frozen shrimp, for 4- and 3-month-old cuttlefish, respectively. In a similar fashion, trypsin activity was 82 ± 5 μg/mg and 80 ± 6 μg/mg of protein, when fed dried shrimp, for 4- and 3-month-old cuttlefish, respectively, and 115 ± 105 μg/mg and 113 ± 9 μg/mg of protein, when fed frozen shrimp, for 4- and 3-month-old cuttlefish, respectively.

Discussion

The main result from the two experiments is that heat treatment of the usual prey for the cuttlefish had a negative effect on the nutritional value of the diet. Similarly, heat treatment at 140°C during varying times (1–24 h) showed an unacceptable loss in the nutritional quality of the protein for heating times longer than 6 h (Márquez et al. 1998). Similarly, it was reported that dry and humid heat on winged bean, *Psophocarpus tetragonolobus*, used to replace fish flowers, did not promote growth of the African catfish, *Clarias gariepinus* (Fagbenro 1999).

Growth rates of cuttlefish obtained in both experiments were smaller when compared with those reported by others for this species at similar temperatures (Domingues et al. 2002, 2003a, b, 2006; Koueta et al. 2002; Koueta and Boucaud-Camou 1999, 2003; Sykes et al. 2006). Nevertheless, these authors report either feeding ad libitum, or at least several times a day. In the present experiments, food was provided only once a day, and remained in the tanks no more than 6 h. Lower feeding rate may explain the smaller growth rates and food conversions obtained, even with high feeding rates for all of the diets tested. In fact, almost all the food offered every day was consumed for all of the diets tested, in both experiments.

A positive correlation between the weight of the digestive gland (DG), and body weight for cuttlefish was reported by Castro and Lee (1994), who suggested that this ratio is a good indicator of the condition of the animal. Results obtained here also indicate a positive correlation between this index and growth rates. The digestive gland represented a higher percentage in relation to body weight when fed the frozen shrimp in both experiments, and the freeze-dried shrimp in experiment 2, which were the diets that promoted growth. This suggests that this relation is indeed a good indicator of animal condition. The correct and constant use of the digestive gland is of extreme importance in cuttlefish growth and condition, since it is the organ of absorption and digestion in *S. officinalis* (Boyle and Rodhouse 2005).

From the early 1990s until the present, feeding experiments with *S. officinalis* have been conducted with either moist or dry pellets (Castro 1990; Lee et al. 1991; Castro et al. 1993) or surimi (fish myofibrillar protein concentrate; Castro et al. 1993; Castro and Lee 1994; Domingues 1999; Domingues et al. 2005). Nevertheless, feeding rates (<3% BW day^{-1}), but especially growth rates on prepared diets have been considerably lower compared with normal laboratory maintenance diet of crustaceans (Pascual 1978; Richard 1971, 1975; Boletzky 1979; Castro et al. 1993; Castro and Lee 1994; Lee et al. 1991; Forsythe et al. 1994; Domingues et al. 2001b, 2002, 2003a, b, 2004; Koueta and Boucaud-Camou 1999, 2001; Koueta et al. 2000). Growth rates were also considerably lower than those obtained during transition periods when cuttlefish were fed thawed catfish fillets (3.0% BW day^{-1}). Similarly, feeding rates obtained here were lower than those for the transition periods that varied between 3.5% and 10% BW day^{-1} (Domingues 1999; Domingues et al. 2005).

Despite the acceptance of the prepared diets, negative growth with artificial diets is common, and the highest growth rates (close to 0.5% BW day^{-1}) reported in the literature (Castro 1990; Castro et al. 1993; Castro and Lee 1994; Lee et al. 1991; Domingues 1999; Domingues et al. 2005) are almost ten times lower than growth rates recorded during normal laboratory maintenance of this species (5% BW day^{-1}) (Pascual 1978; Lee et al. 1991; Forsythe et al. 1994; Domingues et al. 2001b, 2002; Sykes et al. 2003). In addition, mortality rates when feeding artificial diets are usually higher compared to frozen diets (DeRusha et al. 1989; Lee et al. 1991; Castro et al. 1993).

Some authors attributed inferior growth to the lower feeding rates (Castro et al. 1993; Castro and Lee 1994; Domingues et al. 2005); nevertheless, we believed that the negative

or extremely low (<0.5 BW day^{-1}) growth rates obtained could not be explained by the low feeding rates on those diets alone. The present research shows that it was not the low feeding rates with those diets that were responsible for the poor growth. The diets used in those experiments were composed of fish, squid, or other marine organisms that had previously suffered a heating process throughout their preparation, which most likely transformed them into poor bases for the elaboration of artificial diets for the cuttlefish. Fish or squid meals suffer either boiling or drying at high temperatures before being transformed in powder, and the surimi prepared by Castro et al. (1993), Castro and Lee (1994), Domingues (1999) and Domingues et al. (2005) was based on catfish fillets that were cooked before being fed to the cuttlefish. The heat treatment suffered by the shrimp during the current experiments transformed it into a poor diet from the nutritional point of view, probably due to the adulteration, denaturation, and loss (by boiling) of proteins and amino acids. Additionally, the heating processes also oxidize the lipids in a large extent, contributing to the loss of the polar lipids (polyunsaturated fatty acids), which are essential for cephalopods as for other organisms (Navarro and Villanueva 2000; Koueta et al. 2002; Domingues et al. 2003a, b; Almansa et al. 2006). The major difference from previous experiments with artificial diets was that boiled and dried shrimp were consumed at similar rates (even slightly higher) compared with frozen shrimp, contrary to the artificial diets.

Energy absorbed by *S. officinalis* was reduced when shrimp protein was oven-dried or boiled, indicating that some changes in meat characteristics modified the digestive capability of animals. In fact, increases of faeces were recorded in animals fed boiled or oven-dried shrimp, showing that this type of meat was not adequately digested. Previous work on *S. officinalis* showed that diets made with fish meal and supplemented with sardine and squid paste produced similar results (high production of faeces and low absorption efficiency), showing that when protein sources were modified (as the fish meal) assimilation efficiency was limited (Domingues 2007a). The reason for that limited digestibility of processed protein could be related to changes in protein structure. Although there are some unsolved questions regarding protein structure and digestibility capabilities of cephalopods, evidence suggests that processed protein such as fish surimi paste (Castro et al. 1993), catfish processed paste (Domingues et al. 2005) or fish meal enriched with squid paste (Domingues et al. 2007a; Rosas et al. 2007) limit the digested energy in cephalopods.

Digestion capability is related to digestive enzyme activity. The present results show the capacity of *S. officinalis* juveniles to adjust their digestive enzymes to different types of food, and this appears to be well correlated with cuttlefish growth and absorption efficiency. Also, the digestive adaptation could involve different reactions, depending on whether the food satisfies the nutritional requirement or not. Van Wormhoudt (1980) showed that digestive enzymes can be inducted as a response related to growth, or in an attempt to obtain more nutrients from a nutritionally deficient meal. Results obtained in the present study show that trypsin from the digestive gland were well correlated with growth rates, regardless of the age of cuttlefish (3 or 4 months old). A low activity was observed in animals fed boiled or dried shrimp, whereas a high activity was present in cuttlefish fed freeze-dried and frozen shrimp. This suggests that processes involved in digestive enzyme production are related to the cuttlefish's capacity to recognize the nutritional characteristics of the ingested food. There are internal and external stimuli that regulate the activity of the digestive tract of *O. vulgaris* (Best and Wells 1983). Presence of food is by itself enough to stimulate activity in the gut; when an octopus sees food, the digestive gland begins to secrete, initiating a nervous stimulus directed to enzyme production. These authors also observed that salivary glands are stimulated at the same time, producing enzymes that are

used to initiate the external digestion. Best and Wells (1983, 1984) classified these reactions as external stimuli. Additionally, with external stimuli, the presence of food in the crop invoked activity in the salivary glands, suggesting that there are mechanical stimuli in the crop that regulate the salivary enzyme production. According to the same authors, saliva production was independent of the type of food because it was produced even when cellulose was used as inert food. It is interesting to note that they observed that cellulose did not stimulate activity in the digestive gland, indicating that the chemical characteristics of the meal could regulate the secretion of enzymes in the form of proteinaceous "boules". For *S. officinalis*, a similar mechanism could induct the enzymes in the digestive gland. In animals fed boiled or dried shrimp, due to the nutritional deficiency of that diet, crop could not send the chemical signals to adequately stimulate the digestive gland, which responded with a reduction in trypsin production in the digestive gland. The opposite mechanism occurred with acid phosphatases; the chemical signal sent by the crop may have increased enzyme production and enhanced digestibility of the diet. Also, results showed that there were no differences in enzyme activity in animals of different ages (3 and 4 months old).

Current results suggest that enzyme capacity of cuttlefish and, consequently, food digestibility could be improved using nutrients that stimulate enzyme secretion. Differences in induction related with the type of enzyme were observed; while trypsin was inducted in animals with high growth rates, intracellular enzymes (acid phosphatases and acid proteases) were inducted in animals with low growth rates.

On the contrary, a nutritionally adequate diet (freeze-dried or frozen shrimp) could send chemical signals to the digestive gland to induce enzyme production that allowed high assimilation of nutrients and high growth. In contrast, inductions on general acid proteases and acid phosphatases were observed in animals fed boiled and dried shrimp and indicated that with such diets produce and/or activate these type of enzymes.

In conclusion, future research with artificial diets should not be based on fish or marine processed products (such as fish meal) involving any heating procedures during preparation of diets. It is possible that denaturalization of protein or change in protein structure affects the protein in the diet, which reduces its digestibility and in turn the growth rate of cephalopods. Other solutions, such as using hydrolyzed (with high free amino acids or dipeptide concentrations) or freeze-dried products, must be investigated.

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