ELSEVIER

Combined effects of temperature and body weight on growth and protein utilization of the common octopus, *Octopus vulgaris*

Helen Miliou[b], Myrsini Fintikaki[a], Triantaphyllos Kountouris[a], George Verriopoulos[a,*]

[a]Department of Zoology–Marine Biology, Faculty of Biology, School of Sciences, National and Kapodistrian University of Athens, Panepistiomopolis, 157 84 Athens, Greece
[b]Laboratory of Applied Hydrobiology, Faculty of Animal Production, Agricultural University of Athens, Iera Odos 75, 11 855 Athens, Greece

Abstract

Octopus vulgaris is considered a serious candidate for rearing, in terms of its biological and market potential: easy adaptation to captivity conditions, high growth rate and high market price. The aim of this study was to examine the combined effects of temperature and body weight on specific growth rate (SGR, % day^{-1}), absolute growth rate (AGR, g day^{-1}), absolute feeding rate (AFR, g day^{-1}), feed efficiency (FE, %), protein retention efficiency (PRE: [g protein gain g protein ingested^{-1}] × 100), energy retention efficiency (ERE: [kJ gain kJ ingested^{-1}] × 100), proximate composition and *P/E* ratio (protein/energy) of the common octopus. The experiments were performed in a closed system with controlled temperature (15, 20 and 25 °C). The octopuses were fed on squids (*Loligo vulgaris*). In the multiple regression equations estimated for SGR, AGR, AFR, FE, PRE and ERE, the linear and quadratic effects of temperature, as well as the linear effects of body weight were significant. In addition, the interaction effects of temperature and body weight were significant for SGR, AGR, FE, PRE and ERE; the temperature for maximum response decreased with increasing size, being 25 °C for octopuses of 50–150 g and 15 °C for animals of 200–600 g. The weight exponent in the relationships of these indices vs. body weight decreased with increasing temperature. The levels of SGR, FE, PRE and ERE varied slightly with temperature in individuals of 150–200 g, and the respective response surfaces showed a plateau within this range of body sizes independently of temperature. The temperature for maximum AFR was estimated to be 21.88 °C independently of body weight. Proximate composition and *P/E* ratio of octopuses were not affected by temperature or body weight. It is concluded that temperature should be adjusted according to the body weight of *O. vulgaris* for optimizing the economic viability of rearing. Temperature should be high for small individuals (50–150 g) and should be reduced gradually in order for animals larger than 200 g to be reared at low temperature. Therefore, *O. vulgaris* should be reared in closed systems with temperature control.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Octopus vulgaris; Temperature; Body weight; Growth; Feed efficiency; Protein utilization

* Corresponding author. Tel.: +30 210 7274608; fax: +30 210 7274608 (4).
E-mail address: gverriop@biol.uoa.gr (G. Verriopoulos).

1. Introduction

Octopus vulgaris is considered a serious candidate for rearing, in terms of its biological and market potential (Vaz-Pires et al., 2004). It is characterized by high fecundity (Wells, 1978), fast growth (Mangold, 1983; Iglesias et al., 1997) and high feed efficiency (Mangold and von Boletzky, 1973). Other advantages for the aquacultural potential of this species are easy adaptation to captivity conditions, acceptance of low-value natural foods and high market price (Vaz-Pires et al., 2004). Common octopus ongrowing has recently begun to develop in Spanish coastal waters (Iglesias et al., 1997; García García and Aguado Giménez, 2002). The assessment of growth and nutrient utilization of *O. vulgaris* in relation to temperature and size is very important for efficient production management.

The combined effects of temperature and size on growth have been described for several fish species (Fonds et al., 1992; Buckel et al., 1995; Hallaråker et al., 1995; Émsland et al., 1996). It has been shown that, in some fishes, there were shifts in temperature preferenda towards lower temperatures for larger fish (McCauley and Huggins, 1979), and that temperature preferenda of the fish tend to coincide with the optimal temperature for growth (Hill and Magnuson, 1990; Gadomski and Caddell, 1991). Katsanevakis and Verriopoulos (2004) found that octopuses, *Octopus vulgaris*, larger than 200 g tended to dwell deeper during the period of intense thermocline (late spring–summer) in the Eastern Mediterranean, while smaller octopuses preferred warmer temperatures. Thus, a downshift in the temperature for maximum growth with increasing body weight of octopuses could be expected. Aguado Giménez and García García (2002) kept octopuses, *O. vulgaris*, of different size in tanks with open seawater flow and found that the interaction of temperature and size is not significant. It appears that further research is required on growth performance and the nutritional requirements of the common octopus in relation to temperature and size.

O. vulgaris is a carnivorous species and protein is its principal energy source. Cephalopod lipid digestibility is very poor (O'Dor et al., 1984), and their capacity to catabolize lipids is limited (Mommsen and Hochachka, 1981; Navarro and Villanueva, 2000). The common octopus is characterized by a low O/N ratio (oxygen consumption/ammonia excretion) indicative of a protein-dominated catabolism, while an increased O/N ratio suggests that *O. vulgaris* uses up other reserves, such as lipids or carbohydrates under starvation or stress conditions, respectively (O'Dor et al., 1984; Boucher-Rodoni and Mangold, 1985). Aguado Giménez and García García (2002) referred that the growth rate and feed efficiency estimated at various temperatures differed in *O. vulgaris* fed on different diets and suggested that the *P/E* ratio (protein/energy) of the diets is indicative of the variations in these variables. However, protein and energy utilization have not been studied in *O. vulgaris* and, moreover, in relation to temperature and size.

The present study was conducted in order to investigate the combined effects of temperature and body weight on growth rate, food intake, feed efficiency, protein retention efficiency, energy retention efficiency, proximate composition and *P/E* ratio in the common octopus, *O. vulgaris*, maintained in a closed system. The main objective was to develop mathematical models for predicting the aforementioned indices in octopuses of different size at different temperatures. This is important for production management in Mediterranean conditions, where the temperature range is wide.

2. Materials and methods

The octopuses, *O. vulgaris* Cuvier, 1797 (order Octopoda, suborder Incirrata), were collected by free diving in the Saronicos Gulf (Aegean Sea, Eastern Mediterranean) from February 2002 to November 2002. The animals were transferred to the laboratory immediately after collection (within 1 h). No mortality was observed, even in summer, although it has been mentioned that survival of *O. vulgaris* during transportation is inversely related to temperature (Aguado et al., 2001). The octopuses were held indoor at the National and Kapodistrian University of Athens. Experiments were performed at three temperatures (15, 20 and 25 °C), which represent the temperature range in shallow areas of the Saronicos Gulf according to Siokou-Frangou et al. (1993). Diurnal variation in water temperature was ± 0.5 °C. Photoperiod was set at 12 h light: 12 h dark. Before starting of the experiments, the octopuses spent an acclimation period of 30 days.

The holding tanks (capacity 40 or 100 l) were connected to a closed system. The sea water was recycled through mechanical and biological filters and flowed into a reservoir of relatively increased dynamic energy, which served to supply all the holding tanks with recycled sea water (UV-treated and oxygenated) by a constant natural flow. In each holding tank, the inflow was kept constant and the seawater was renewed seven times per day. Salinity (37.5–38.5‰), dissolved oxygen (5.8–6.3 mg l^{-1}) and pH (7.8–8.2) were controlled daily. For nitrites and un-ionized ammonia, the concentrations measured (twice weekly) were <0.1 and <0.05 mg l^{-1}, respectively.

The octopuses were kept apart during the acclimation and experimental period, because of the high degree of cannibalism. Furthermore, isolation allows reliable determination of growth and food intake. Specimens were handfed on squids (*Loligo vulgaris*), once a day, at midday during the acclimation and experimental period. Previously, batches of squids were frozen to be used as food supply. The ration was 3% of body weight per day and was in excess for all conditions. Animals were adapted to this ration in a few days after collection from the sea and were weighed individually, every 10 days, for adjusting the amount of food provided. Any remaining food was removed within 10 min, drained and weighed. Ingested food was the difference between supplied food and remaining food. Prior to weighing, octopuses were deprived of food for 24 h.

The experimental period lasted 30 days for each octopus. A total of 50 octopuses were used (16–17 specimens at each temperature), with initial body weight ranging from 40 to 621.5 g and final body weight from 56 to 773 g. At the end of the experiment, each octopus was weighed, placed on ice and died immediately. For each animal, the sex and the stage of maturity were determined according to the descriptions given by Nigmatulin (1977) for the six stages of maturity in males and females, the two last stages being the mature and post-spawning ones. Then, each octopus was homogenized (after removal of the viscera) and freeze-dried. The same procedure was followed for samples from each batch of frozen squids.

Proximate composition was determined for each octopus ($n = 50$) and each batch of squids ($n = 3$), and all chemical analyses were performed in triplicate. Dry matter was obtained by drying in an oven at 105 °C for 24 h, and ash by incineration in a muffle furnace at 550 °C for 24 h. Crude protein (nitrogen × 6.25) was determined by the method described by Hach et al. (1985). Lipids were extracted according to the method of Folch et al. (1957). Gross energy was estimated using the Miglavs and Jobling (1989) energy coefficients: protein 23.6 kJ and lipid 38.9 kJ. Proximate composition of squids did not vary among the three batches, and the mean values of the macronutrient contents are given in Table 1.

Specific growth rate (SGR, % day^{-1}) was calculated as $SGR = 100 (\ln W_t - \ln W_0)t^{-1}$, where W_t = final weight (g), W_0 = initial weight (g), and t the experimental duration. Absolute growth rate (AGR, g day^{-1}) was calculated as $AGR = (W_t - W_0)t^{-1}$. Absolute feeding rate (AFR, g day^{-1}) was calculated as $AFR = FI t^{-1}$, where FI was the cumulative food intake (g). Feed efficiency (FE, %) was calculated as: $FE = 100 (W_t - W_0) FI^{-1}$, where W_t, W_0 and FI were

Table 1
Proximate composition (% wet weight) and *P/E* (g protein/MJ) of the common squid and the common octopus reared at three temperatures (S.E.M.: standard error of grand mean)

	Loligo vulgaris		*Octopus vulgaris*					
	$n=3$	S.E.M.	15 (°C) $n=16$	20 (°C) $n=17$	25 (°C) $n=17$	S.E.M.	*F*	*P*
Dry matter	17.72	0.079	18.89	18.94	18.73	0.072	0.75	n.s.
Crude protein	13.82	0.002	15.27	15.23	15.00	0.073	1.36	n.s.
Crude lipid	1.86	0.042	0.82	0.81	0.83	0.003	2.24	n.s.
Ash	0.95	0.020	2.00	1.99	1.97	0.009	0.47	n.s.
P/E	34.68		38.93	38.96	38.83			

n.s.: not significant ($P > 0.05$).

determined in grams on a dry-matter basis. Protein retention efficiency (PRE, %) was calculated (g protein gain g protein ingested^{-1}) according to the formula PRE=100 [($P_t W_t - P_0 W_0$) (P_fFI)$^{-1}$], where P_0 and P_t were the initial and final protein concentrations of the octopus (% dry weight), P_f was the protein concentration of the food (% dry weight), and W_t, W_0 and FI were determined in grams on a dry-matter basis. Energy retention efficiency (ERE, %) was calculated (kJ gain kJ ingested^{-1}) according to the formula: ERE=100 [($GE_t W_t - GE_0 W_0$) (GE_fFI)$^{-1}$], where GE_0 and GE_t were the initial and final gross energy concentrations of the octopus (% dry weight), GE_f was the gross energy of the food (% dry weight), and W_t, W_0 and FI were determined in grams on a dry-matter basis (Bendiksen et al., 2003).

Pre−experimental trials (in the same closed system, under the same feeding conditions, at 20 °C) showed that the growth rate and proximate composition of common octopus (estimated in around 10 animals every 30 days) were not affected significantly by the time of rearing (210 days after collection from the sea). In addition, body weight affected growth rate but not proximate composition. The definition of the acclimation and experimental period used in the present study was based on the aforementioned results. These also permitted the calculation of protein and energy retention efficiency, by assuming that the initial concentration of macronutrients is similar to the final one; determination of the former was impossible due to individual measurements.

As the specimens were collected from the sea, it was impossible to obtain replicates of the same body weight or even to form groups of animals having a narrow range of body weight. The following regression equation was used to describe the relationship between SGR, AGR, AFR, FE, PRE or ERE (Y) and body weight at each temperature: $\ln Y = a + b \ln W$, where W is the geometric mean weight (g) of the common octopus calculated as $0.5(W_t + W_0)$. The significance ($P < 0.05$) of the differences among the three regression coefficients, as well as among the three intercepts, was tested (ANCOVA) for all the abovementioned indices. The relationship between the content (% weight) in dry matter, crude protein, crude lipid or ash vs. body weight was not significant at any temperature, and ANOVA (Tukey multiple range test)

was performed for comparing the means among the three temperatures.

The following model was used to describe the effects of body weight (W), temperature (T, °C) and sex (S) on SGR, AGR, AFR, FE, PRE and ERE (Y): $\ln Y = \ln a + b_1 \ln W + b_2 T \ln W + b_3 T + b_4 T^2 + b_5 S$. The quadratic effects of body weight were not included in the model, since a natural-log relationship between body weight and growth or food intake has been clearly established for most animals. The significance of the independent variables $T \ln W$, T^2 and S varied among the models developed for explaining variations in growth and food intake (Wooton et al., 1980; Liu et al., 1998; Lupatsch and Kissil, 1998; García García and Aguado Giménez, 2002). Sex is a qualitative variable and was encoded as $S=0$ for males and $S=1$ for females. Multiple regression analysis was used to calculate the parameters of the model, and backward elimination procedure was performed to remove any variables that were not significant. ANOVA was used to test the significance of models and Student's t-test for the coefficient significance. A variable was removed only if the nominal significance of its partial regression coefficient was $P > 0.1$ (Sokal and Rohlf, 1995). When the above model was described by a parabolic regression, then the temperature for maximum response was calculated considering that $dY/dT=0$.

Normality of distributions was assessed by the Kolmogorov–Smirnov test, homogeneity of variances was tested using the Bartlett's test and independence of errors was checked by the Durbin–Watson index values (Sokal and Rohlf, 1995; Zar, 1996). In cases of non-significant statistical tests, power ($1 - \beta$) analysis for those tests were performed according to the methods described by Searcy-Bernal (1994) and Zar (1996), using $\alpha = 0.05$. All statistical analyses were performed with the computer Statgraphics plus 4 software.

3. Results

Proximate composition of *O. vulgaris* did not vary significantly with temperature (Table 1), as it was proved by a comparison (ANOVA) of dry matter, crude protein, crude lipid and ash (% wet weight) estimated at the three temperatures ($P > 0.05$, power

$(1-\beta)>0.8)$. As a result, the P/E ratio of the common octopus was similar at the three temperatures.

The results of specific growth rate (SGR, % day^{-1}), absolute growth rate (AGR, g day^{-1}), absolute feeding rate (AFR, g day^{-1}), feed efficiency (FE, %), protein retention efficiency (PRE, %) and energy retention efficiency (ERE, %) in relation to mean body weight (W, g) are given graphically in Fig. 1. The power function, $\ln Y = a + b \ln W$, described the relationships SGR vs. W (Eqs. (1)–(3)), AGR vs. W (Eqs. (4)–(6)), AFR vs. W (Eqs. (7)–(9)), FE vs. W (Eqs. (10)–(12)), PRE vs. W (Eqs. (13)–(15)) and
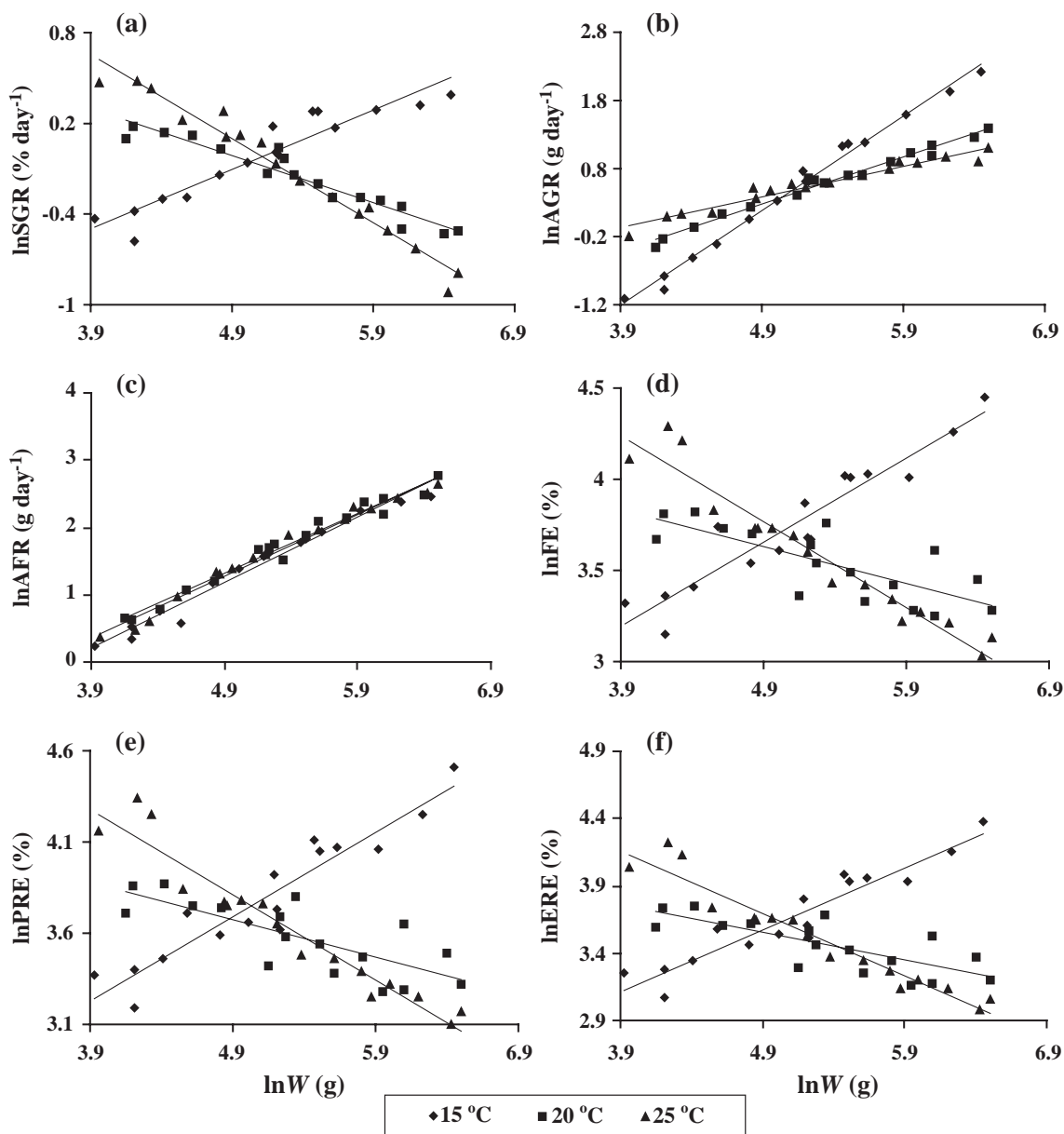

Fig. 1. Regression curves of SGR (a), AGR (b), AFR (c), FE (d), PRE (e) or ERE (f) vs. body weight (W) at three temperatures.

ERE vs. W (Eqs. (16)–(18)) at each temperature (Table 2). All the estimated linear regression equations were highly significant ($P<0.001$). As body weight increased, SGR, FE, PRE and ERE decreased at 20 °C (b values ranged from -0.2020 to -0.3106) and moreover at 25 °C (b values ranged from -0.4673 to -0.5842), but increased at 15 °C (b values ranged from 0.3936 to 0.4638). AGR increased with weight, but at a higher rate at 15 °C ($b=1.3880$) than at 20 °C ($b=0.6912$) and 25 °C ($b=0.4219$).

ANCOVA showed significant differences among the intercepts ($P<0.01$) and slopes ($P<0.0001$) of the three linear relationships of SGR, AGR, FE, PRE or ERE vs. mean body weight (Fig. 1a, b, d, e and f, respectively). The intercepts varied significantly ($P<0.05$), but not the slopes ($P>0.05$, power $(1-\beta)>0.9$), among the three AFR vs. W relationships (Fig. 1c).

Table 3 shows results of multiple regression analyses. ANOVA proved that all models are highly significant ($P<0.0001$). Multiple regression analysis (backward elimination procedure) showed that the following models described the effects of body weight

(W, g) and temperature (T, °C) on SGR, AGR, FE, PRE and ERE:

$$\ln SGR = -7.1930 + 1.7096\ln W - 0.0933T\ln W + 0.3087T + 0.0042T^2 \tag{19}$$

$$\ln AGR = -11.7464 + 2.6850\ln W - 0.0920T\ln W + 0.3090T + 0.0041T^2 \tag{20}$$

$$\ln AFR = -4.4608 + 0.9272\ln W + 0.1138T - 0.0026T^2 \tag{21}$$

$$\ln FE = -2.7115 + 1.7646\ln W - 0.0917T\ln W + 0.2003T + 0.0065T^2 \tag{22}$$

$$\ln PRE = -2.6701 + 1.7571\ln W - 0.0913T\ln W + 0.2013T + 0.0065T^2 \tag{23}$$

$$\ln ERE = -2.7650 + 1.7613\ln W - 0.0914T\ln W + 0.1971T + 0.0066T^2 \tag{24}$$

In the multiple regression equations estimated for all the aforementioned parameters, the linear and qua-

Table 2
Relationships between body weight (W, g) and SGR (% day^{-1}), AGR (g day^{-1}), AFR (g day^{-1}), FE (%), PRE (%) or ERE (%) at different temperatures

T (°C)	Regression equation	n	R^2	P	
15	$\ln SGR = -2.0342 + 0.3936\ln W$	16	89.51	***	(1)
20	$\ln SGR = 1.5091 - 0.3106\ln W$	17	91.92	***	(2)
25	$\ln SGR = 2.9832 - 0.5842\ln W$	17	97.59	***	(3)
15	$\ln AGR = -6.6206 + 1.3880\ln W$	16	99.09	***	(4)
20	$\ln AGR = -3.1116 + 0.6912\ln W$	17	98.25	***	(5)
25	$\ln AGR = -1.6626 + 0.4219\ln W$	17	95.20	***	(6)
15	$\ln AFR = -3.5493 + 0.9662\ln W$	16	97.03	***	(7)
20	$\ln AFR = -3.0741 + 0.8955\ln W$	17	97.35	***	(8)
25	$\ln AFR = -3.2478 + 0.9229\ln W$	17	97.82	***	(9)
15	$\ln FE = 1.3911 + 0.4618\ln W$	16	90.01	***	(10)
20	$\ln FE = 4.6212 - 0.2020\ln W$	17	57.93	**	(11)
25	$\ln FE = 6.1824 - 0.4897\ln W$	17	93.68	***	(12)
15	$\ln PRE = 1.4248 + 0.4622\ln W$	16	89.08	***	(13)
20	$\ln PRE = 4.6789 - 0.2051\ln W$	17	58.33	**	(14)
25	$\ln PRE = 6.1936 - 0.4837\ln W$	17	92.76	***	(15)
15	$\ln ERE = 1.2986 + 0.4638\ln W$	16	89.40	***	(16)
20	$\ln ERE = 4.5462 - 0.2028\ln W$	17	57.22	**	(17)
25	$\ln ERE = 5.9837 - 0.4673\ln W$	17	93.40	***	(18)

***$P<0.0001$.
**$P<0.001$.

Table 3
Results of multiple regression analyses $\ln Y = \ln a + b_1 \ln W + b_2 T \ln W + b_3 T + b_4 T^2$, where Y: SGR, AGR, AFR, FE, PRE or ERE ($\ln a$, $b_1 - b_4$: coefficients; S.E.: standard error; S.E.E.: standard error of estimation)

	$\ln a$ S.E. P	b_1 S.E. P	b_2 S.E. P	b_3 S.E. P	b_4 S.E. P	R^2 adjusted (%) S.E.E.	ANOVA F P	
SGR	−7.1930 0.7791 ***	1.7096 0.1158 ***	−0.0933 0.0056 ***	0.3087 0.0623 ***	0.0042 0.0014 *	87.73 0.1184	88.62 ***	(19)
AGR	−11.7464 0.7796 ***	2.6850 0.1159 ***	−0.0920 0.0056 ***	0.3090 0.0623 ***	0.0041 0.0014 *	96.91 0.1185	385.49 ***	(20)
AFR	−4.4608 0.5464 ***	0.9272 0.0227 ***	n.s.	0.1138 0.0564 *	−0.0026 0.0014 †	97.26 0.1169	579.83 ***	(21)
FE	−2.7115 0.8944 *	1.7646 0.1329 ***	−0.0917 0.0064 ***	0.2003 0.0715 *	0.0065 0.0016 **	82.86 0.1360	60.24 ***	(22)
PRE	−2.6701 0.9213 *	1.7571 0.1369 ***	−0.0913 0.0066 ***	0.2013 0.0737 *	0.0065 0.0017 **	81.78 0.1401	55.97 ***	(23)
ERE	−2.7650 0.9178 *	1.7613 0.1364 ***	−0.0914 0.0066 ***	0.1971 0.0734 *	0.0066 0.0017 **	81.92 0.1395	56.52 ***	(24)

***$P<0.0001$. *$P<0.05$. †$P<0.1$. **$P<0.001$. n.s.: not significant ($P>0.1$).

dratic effects of temperature, as well as the linear effects of body weight, were significant ($P<0.1$). In SGR, AGR, FE, PRE and ERE models, the joint effect of temperature and weight ($T\ln W$) was highly significant ($P<0.0001$), indicating that the weight exponent b in SGR vs. W, AGR vs. W, FE vs. W, PRE vs. W and ERE vs. W relationships was a function of temperature. In AFR model, interaction of temperature and body weight ($T\ln W$) was not significant ($P>0.1$), indicating that AFR increased with body weight independently of temperature. Sex coefficient (S) was not significant ($P>0.1$) in all the aforementioned models, so this variable was removed. The sex was discernable in all specimens, which were immature (up to stage 4).

The effects of temperature and weight on SGR, AGR, AFR, FE, PRE and ERE were described by the response surfaces (Fig. 2) given by the polynomial Eqs. (19)–(24), respectively. The response to temperature changed with size (50–600 g), apart that of AFR. In octopuses weighing 50–150 g, the maximum SGR (2.096–1.058% day^{-1}), AGR (1.028–1.570 g day^{-1}), FE (74.55–41.79%), PRE (77.65–43.65%) and ERE (69.04–38.84%) was estimated at 25 °C, while in animals of 200–600 g the maximum for

SGR (1.046–1.459% day^{-1}), AGR (2.053–8.610 g day^{-1}), FE (45.85–70.36%), PRE (47.50–72.77%) and ERE (42.22–64.83%) was found at 15 °C. The SGR, FE, PRE and ERE levels varied slightly with temperature in octopuses weighing 150–200 g, and the respective response surfaces showed a plateau within this range of body sizes independently of temperature.

Calculated from the models (every 10 g), the temperature for maximum SGR (Eq. (19)) and AGR (Eq. (20)) decreased with increasing size, being 25 °C in octopuses of 50–160 g and 15 °C in animals of 170–600 g. The temperature estimated for maximum FE (Eq. (22)), PRE (Eq. (23)) and ERE (Eq. (24)) was 25 °C in octopuses of 50–150 g, and fell to 15 °C in animals of 160–600 g. The shift in temperature for maximum growth rate was slower than that for maximum FE, PRE and ERE, and the temperature for maximum SGR was higher compared to that for the maximum response of the other indices in a 160-g octopus. The model estimated for AFR (Eq. (21)) was parabolic independently of body weight, and the temperature for maximum response was calculated to be 21.88 °C ($T = -b_3/2b_4$).
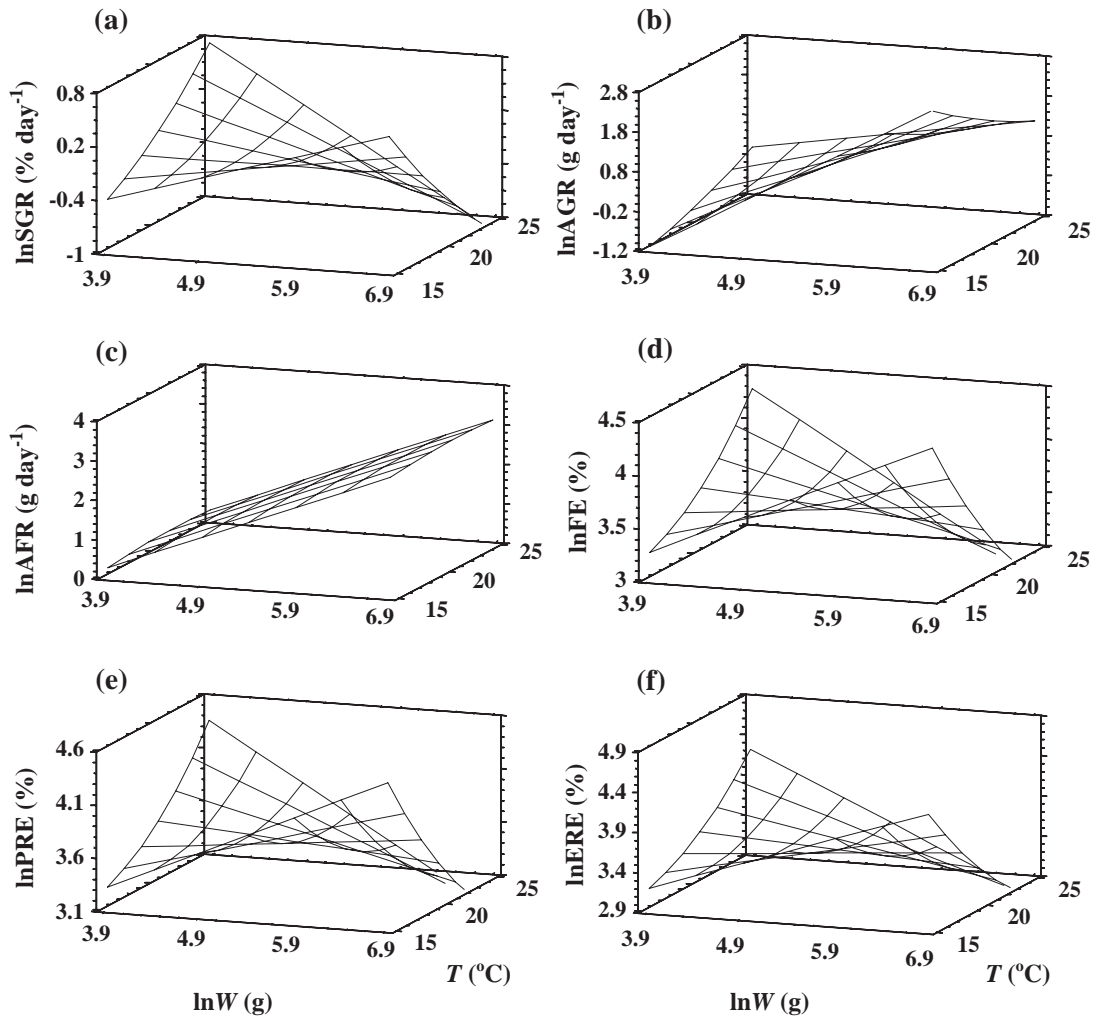
Fig. 2. Response surfaces representing the combined effects of temperature (T) and body weight on SGR (a), AGR (b), AFR (c), FE (d), PRE (e) and ERE (f).

Weight coefficients in linear relationships (b) and models ($b_1 + b_2 T$) were lower for SGR than for FE, PRE and ERE at the respective temperature, indicating that changes in specific growth rate with size were slower compared to those in feed efficiency, protein retention efficiency and energy retention efficiency.

4. Discussion

According to the present results, the temperature for maximum growth in *O. vulgaris* was depended on body weight, being lower for the larger octopuses

(>170 g). The interaction of temperature and body weight on the growth of *O. vulgaris* did not proved significant in a previous study (Aguado Giménez and García García, 2002), probably because the specimens were larger than 170 g. In fish, studies on the interaction between size and temperature for growth have produced inconsistent results. Significant interaction effects between body weight and temperature on growth were found in *Scophthalmus maximus* (Imsland et al., 1996), *Pomatomus saltratrix* (Buckel et al., 1995) and *Channa argus* (Liu et al., 1998). In contrast, interaction of body weight and temperature was not significant for growth in *Casterosteus aculeatus*,

Phoxinus phoxinus (Wooton et al., 1980) and *Siniperca chuatsi* (Liu et al., 1998). The influence of the interaction between body weight and temperature on growth implies an ontogenetic shift in the temperature optimum for growth with increasing fish size (Cuenco et al., 1985; Pedersen and Jobling, 1989; Fonds et al., 1992; Hallaråker et al., 1995; Björnsson and Tryggvadóttir, 1996; Imsland et al., 1996; Liu et al., 1998). Indeed, the downshift in the temperature for maximum growth with increasing size in this study is in accordance with the size distribution of *O. vulgaris* along the coasts of the Eastern Mediterranean (Katsanevakis and Verriopoulos, 2004).

Under the experimental conditions of this study, the temperature for maximum feed efficiency decreased with increasing body weight of *O. vulgaris*. A decline in the temperature for maximum growth rate and feed efficiency with increasing size has also been described for immature halibut, *Hippoglossus hippoglossus*, which was slower for the former resulting in a lower temperature optimum for feed efficiency than for growth rate (Björnsson and Tryggvadóttir, 1996). A tendency for a lower temperature optimum for feed efficiency compared to that for growth rate has also been found for the sockeye salmon, *Oncorhynchus nerka* (Brett et al., 1969), brown trout, *Salmo trutta* (Elliot, 1976) and several other species of fish as reviewed by Jobling (1995). The explanation for this general finding is that at unrestricted ration growth rate reaches a maximum at a temperature lower than that giving maximum ingestion rate (Jobling, 1995). In the present study, the ration was adjusted according to the body weight, and the temperature for maximum food intake was (21.88 °C) near to that (20 °C) previously demonstrated for *O. vulgaris* fed to satiation (Aguado Giménez and García García, 2002). The temperature for maximum feed efficiency tended to be close to that for maximum growth rate. However, growth rate changed with size slower than feed efficiency in a 160-g octopus, resulting in a lower temperature maximum for feed efficiency (15 °C) compared to that for growth rate (25 °C). In that case, the growth rate decreased slightly with decreasing temperature, being higher than the ingestion rate as the temperature lowered below the maximum for food intake. The thermal tolerance observed in octopuses weighing 150–200 g is consistent with the large variation in temperatures experienced by the common octopus during late summer–early autumn (breakdown of thermocline) in the coastal areas of Aegean Sea, when octopuses of 150–200 g are abundant (Katsanevakis and Verriopoulos, 2004).

In the present study, protein utilization was more efficient at the temperature for maximum growth rate according to the body weight of the common octopus; in smaller (50–150 g) individuals, protein retention efficiency was better at 25 °C, while in larger ones (200–600 g) at 15 °C. Kibria et al. (1997) observed that silver perch, *Bidyanus bidyanus*, grown at its optimal temperature retained maximum nitrogen compared with fish reared at other temperatures. Bendiksen et al. (2003) found increased protein retention efficiency and improved growth rate in Atlantic salmon parr, *Salmo salar*, at 8 °C than at 2 °C. In juvenile European sea bass, *Dicentrarchus labrax*, growth rate, feed efficiency and protein digestibility were improved at the higher temperature (25 °C), but protein retention efficiency was higher at the lower temperature (18 °C) when the dietary protein content was less than the one required (Peres and Oliva-Teles, 1999). In addition, energy retention efficiency improved with the increase of dietary protein level at the higher temperature. The increased protein retention efficiency estimated in this study for the smaller octopuses at the higher temperature indicates that the protein content of squid satisfies the requirements of common octopus with respect to amino acids for obtaining energy and for protein synthesis. This was assured by the increased energy utilization in the smaller octopuses at the higher temperature. Furthermore, the P/E ratio was independent of temperature or body weight of the common octopus. The protein content of *O. vulgaris* remained constant within the range of body weight studied and was slightly higher than that given by Villanueva et al. (2004) for the wild juveniles (approximately 15 g) of this species. In accordance with our results, Katsanevakis et al. (2005) found for *O. vulgaris* fed on squids a low O/N ratio, which did not differ significantly with temperature (15–28 °C) or body weight (50–600 g), and concluded that the dietary protein content fulfilled the nitrogen requirements of the common octopus.

The weight exponent (b) in the growth–body weight relationships estimated for *O. vulgaris* tended to decrease with increasing temperature. Similarly, the weight exponent was dependent on temperature in fish species whose optimal temperature for growth varied

with temperature (Wangila and Dick, 1996; Liu et al., 1998), although Jobling (1983) suggested that the *b* value in the growth–fish size relationship is often −0.40. The multiple regression Eqs. (19)–(24) developed in the present study accounted for 81.78–97.26 % of the variations in the dependent variables and can be used to predict the growth rate, food intake, feed efficiency and protein and energy retention efficiency of *O. vulgaris* under specific rearing conditions. However, application of the models to smaller and larger octopuses than those used in the present study needs to be tested. Also, the effects of sexual maturation and reproduction require further consideration, since literature data are contradictory (Wells and Wells, 1959; Mangold, 1963; Smale and Buchan, 1981; García García and Aguado Giménez, 2002). In this study, sex did not affect the aforementioned parameters significantly, probably because all the octopuses were immature.

As with these results concerning the larger octopuses (>170 g) fed on squids, Aguado Giménez and García García (2002) reported higher growth rates at 15 °C than at 25 °C for *O. vulgaris* (175–3450 g) fed on crabs or bogue. In addition at 15 °C, squid-fed octopuses performed a superior growth rate than bogue-fed individuals, but an inferior one compared to crab-fed animals (squid-fed 6.79 g day^{-1}, bogue-fed 5.65 g day^{-1}, crab-fed 12.52 g day^{-1}, simulated for 500-g octopuses). Aguado Giménez and García García (2002) explained the increased growth rate of crab-fed octopuses compared to that of bogue-fed animals by the higher *P/E* ratio estimated for crabs (34.03) than that for bogue (27.70), since the protein content of both diets was similar. In addition, García García and Aguado Giménez (2002) obtained better growth results with bogue than sardine (15.02 *P/E* ratio). Nixon (1966) also reported that octopuses grew better with shrimps or crabs than fish. O'Dor et al. (1984) claimed that the *P/E* optimum for *O. vulgaris* is around 35, as its natural diet is composed mainly of crabs. The higher *P/E* ratio for squid (34.68) than for bogue or sardine might explain the better growth rate and feed efficiency of squid-fed (present study) compared to fish-fed octopuses with a similar food intake at 15 °C (Aguado Giménez and García García, 2002; García García and Aguado Giménez, 2002). However, the *P/E* ratio is similar for crabs and squids, and thus, the improved growth rate of crab-fed compared to squid-fed octo-

puses seems to be associated with the differences in the quantity of the food consumed; food intake at 15 °C for squid-fed octopuses (present study) was lower than that for crab-fed octopuses at the same temperature (Aguado Giménez and García García, 2002), but feed efficiency for the former was much higher than that for the latter (squid-fed 65.53% vs. crab-fed 35%, simulated for 500 g octopuses).

Aguado Giménez and García García (2002) observed weight loss and mortality in *O. vulgaris* reared at temperatures above 21 °C. The same authors (2002) noted that *O. vulgaris* should be reared from 16 to 21 °C and that this range is too narrow, considering the wide natural range (12–29 °C) in some Mediterranean areas. Present results extended the range of temperature for rearing *O. vulgaris* from 15 to 25 °C, as no mortality was observed and feed efficiency of octopuses was higher than 20%. However, temperature should be adjusted according to the body weight of *O. vulgaris* for optimising economic viability. Temperature should be high for small individuals (50–150 g) and should be reduced gradually in order for animals larger than 200 g to be reared at low temperature. Therefore, *O. vulgaris* should be reared in closed systems with temperature control. The present results obtained in a closed system showed satisfactory SGR levels for small (25 °C) and large (15 °C) squid-fed octopuses, which are similar to the SGR levels estimated (1.6 ± 0.30% day^{-1} for 50–200 g and 1.19 ± 0.31% day^{-1} for 200–500 g) for *O. vulgaris* in its natural habitat (Katsanevakis, 2004).

Acknowledgements

References

Aguado Giménez, F., García García, B., 2002. Growth and food intake in *Octopus vulgaris* Cuvier (1997): Influence of body weight, temperature, sex and diet. Aquac. Int. 10, 361–377.

Aguado, F., Rueda, F.M., Egea, M.A., Hernández, M.D., Faraco, F., García García, B., 2001. Effecto de la temperatura sobre la supervivencia en el transporte, estabulación y engorde de *Octopus vulgaris* Cuvier (1797) en el Mediterráneo occidental. In: Fernández-Palacios, H., Izquierdo, M. (Eds.), Convergencia entre Investigación y Empresa: Un reto para el Siglo XXI. Serie Monografías del ICCM, vol. 4. Las Palmas de Gran Canaria, pp. 174–179.

Bendiksen, E.Å., Berg, O.K., Jobling, M., Arnensen, A.M., Måsøval, K., 2003. Digestibility, growth and nutrient utilization of Atlantic salmon parr (*Salmo salar* L.) in relation to temperature, feed fat content and oil source. Aquaculture 224, 283–299.

Björnsson, B., Tryggvadóttir, S.V., 1996. Effects of size on optimal temperature for growth and growth efficiency of immature Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture 142, 33–42.

Boucher-Rodoni, R., Mangold, K., 1985. Ammonia excretion during feeding and starvation in *Octopus vulgaris*. Mar. Biol. 86, 193–197.

Brett, J.R., Shelbourn, J.E., Shoop, C.T., 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. J. Fish. Res. Board Can. 26, 2363–2394.

Buckel, J.A., Steinberg, N.D., Conover, D.O., 1995. Effects of temperature, salinity, and fish size on growth and consumption of juvenile bluefish. J. Fish Biol. 47, 696–706.

Cuenco, M.L., Stickney, R.R., Grant, W.E., 1985. Fish bioenergetics and growth in aquaculture ponds: Effects of interactions among size, temperature, dissolved oxygen, unionized ammonia and food on growth of individual fish. Ecol. Model. 27, 191–206.

Elliot, J.M., 1976. The energetics of feeding, metabolism and growth of brown trout (*Salmo trutta* L.) in relation to body weight, water temperature and ration size. J. Anim. Ecol. 45, 923–948.

Folch, J., Lees, N., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Fonds, M., Cronie, R., Vethaak, A.D., van der Puyl, P., 1992. Metabolism, food consumption and growth of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) in relation to fish size and temperature. Neth. J. Sea Res. 29, 127–143.

Gadomski, D.M., Caddell, S.M., 1991. Effects of temperature on early-life-history stages of California halibut *Paralichthys californicus*. Fish. Bull. 89, 567–576.

García García, B., Aguado Giménez, F., 2002. Influence of diet on ongrowing and nutrient utilization in the common octopus (*Octopus vulgaris*). Aquaculture 211, 171–182.

Hach, C.C., Brayton, S.V., Kopelove, A.B., 1985. A powerful Kjeldahl Nitrogen method using peroxymonosulfuric acid. J. Agric. Food Chem. 33, 1117–1123.

Hallaråker, H., Folkvord, A., Stefansson, S.O., 1995. Growth of juvenile halibut (*Hippoglossus hippoglossus*) related to temperature, light period and feeding regime. Neth. J. Sea Res. 34, 139–147.

Hill, D.K., Magnuson, J.J., 1990. Potential effects of global climate warming on the growth and prey consumption of Great Lakes fish. Trans. Am. Fish. Soc. 119, 265–275.

Iglesias, J., Sánchez, F.J., Otero, J.J., 1997. Primeras experiencias sobre el cultivo integral del pulpo (*Octopus vulgaris*, Cuvier) en el Instituto Español de Oceanografía. In: de Costa, J., Abellán, E., García García, B., Ortega, A., Zamora, S. (Eds.), Actas VI Congresso Nacional de Acuicultura, Cartagena 9–11 de Julio de 1997. Ministerio de Agricultura, Pesca y Alimentación, Madrid, pp. 221–226.

Imsland, A.K., Sunde, L.M., Folkvord, A., Stefansson, S.O., 1996. The interaction of temperature and fish size of juvenile turbot. J. Fish Biol. 49, 926–940.

Jobling, M., 1983. Growth studies with fish—overcoming the problems of size variation. J. Fish Biol. 22, 153–157.

Jobling, M., 1995. The influence of environmental temperature on growth and conversion efficiency in fish. ICES C.M. 1995, 4.

Katsanevakis, S. 2004. Ecology of *Octopus vulgaris*. PhD thesis, National and Kapodistrian University of Athens, Greece.

Katsanevakis, S., Verriopoulos, G., 2004. Abundance of *Octopus vulgaris* on soft sediment. Sci. Mar. 68, 553–560.

Katsanevakis, S., Stephanopoulou, S., Miliou, H., Moraitou-Apostolopoulou, M., Verriopoulos, G., 2005. Oxygen consumption and ammonia excretion of *Octopus vulgaris* (Cephalopoda) in relation to body mass and temperature. Mar. Biol. 146, 725–732.

Kibria, G., Negegoda, D., Fairclough, R., 1997. Nitrogen pollution from aquaculture: Nitrogen losses and nitrogen retention by silver perch *Bidyanus bidyanus* (Mitchell, 1838) (Teraponidae). Joint Conference of Australian Society for Fish Biology (ASFB) and Fish and Aquatic Resource Management Association of Australia (FARMAA), 15–19 July, Darwin, Northern Territory, Australia. Abstract.

Liu, J., Cui, Y., Liu, J., 1998. Food consumption and growth of two piscivorous fishes, the Mandarin fish and Chinese snakehead. J. Fish Biol. 53, 1071–1083.

Lupatsch, I., Kissil, G.W., 1998. Predicting aquaculture waste from gilthead seabream (*Sparus aurata*) culture using a nutritional approach. Aquat. Living Resour. 4, 265–268.

Mangold, K., 1963. Biologie de Céphapodes benthiques et nectoniques de la Mer Catalane. Vie et Milleu, 1–33.

Mangold, K.M., 1983. *Octopus vulgaris*. In: Boyle, P.R. (Ed.), Cephalopod Life Cycles, vol. 1. Academic Press, London, pp. 335–364.

Mangold, K.M., von Boletzky, S.V., 1973. New data on reproductive biology and growth of *Octopus vulgaris*. Mar. Biol. 19, 7–12.

McCauley, R.W., Huggins, N.W., 1979. Ontogenetic and non-thermal seasonal effects on thermal preferenda of fish. Am. Zool. 19, 267–271.

Miglavs, I., Jobling, M., 1989. The effects of feeding regime on proximate body composition and patterns of energy deposition in juvenile arctic charr, *Salvelinus alpinus*. J. Fish Biol. 35, 1–11.

Mommsen, T.P., Hochachka, P.W., 1981. Respiratory and enzymatic properties of squid heart mitochondria. Eur. J. Biochem. 120, 345–350.

Navarro, J.C., Villanueva, R., 2000. Lipid and fatty acid composition of early stages of cephalopods: An approach to their lipid requirements. Aquaculture 183, 161–177.

Nigmatulin, CH.M., 1977. Proisvodstvo i biologuicheskikh nabludéni v more i ikh obrabotka i oformlénié. Kalingrad, 56–78.

Nixon, M., 1966. Changes in body weight and intake of food by *Octopus vulgaris*. J. Zool. Lond. 150, 1–9.

O'Dor, R.K., Mangold, K., Boucher-Rodoni, R., Wells, M.J., Wells, J., 1984. Nutrient absorption, storage and remobilization in *Octopus vulgaris*. Mar. Behav. Physiol. 11, 239–258.

Pedersen, T., Jobling, M., 1989. Growth rates of large, sexually mature cod, *Gadus morhua*, in relation to condition and temperature during an annual cycle. Aquaculture 81, 161–168.

Peres, H., Oliva-Teles, A., 1999. Influence of temperature on protein utilization in juvenile European seabass (*Dicentrarchus labrax*). Aquaculture 170, 337–348.

Searcy-Bernal, R., 1994. Statistical power and aquacultural research. Aquaculture 127, 371–388.

Siokou-Frangou, I., Verriopoulos, G., Yannopoulos, C., Moraitou-Apostolopoulou, M., 1993. Differentiation of zooplankton communities in two neighbouring shallow areas. In: Eleftheriou, A. (Ed.), Biology and Ecology of Shallow Coastal Waters, EMBS Symposium, vol. 28. Olsen and Olsen, Denmark, pp. 87–97.

Smale, M.J., Buchan, P.R., 1981. Biology of *Octopus vulgaris* off the East coast of South Africa. Mar. Biol. 65, 1–12.

Sokal, R.R., Rohlf, F.S., 1995. Biometry, 3rd edition. W.H. Freeman and Company, New York. 887 pp.

Vaz-Pires, P., Seixas, P., Barbosa, A., 2004. Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): A review. Aquaculture 238, 221–238.

Villanueva, R., Riba, J., Ruíz-Capillas, C., González, A.V., Baeta, M., 2004. Amino acid composition of early stages of cephalopods and effect of amino acid dietary treatments on *Octopus vulgaris* paralarvae. Aquaculture 242, 455–478.

Wangila, B.C.C., Dick, T.A., 1996. Influence of genotype and temperature on the relationship between specific growth rate and size of rainbow trout. Trans. Am. Fish. Soc. 117, 560–564.

Wells, M.J., 1978. Octopus. Physiology and Behaviour of an Advanced Invertebrate. Chapman & Hall, London. 417 pp.

Wells, M.J., Wells, J., 1959. Hormonal control of sexual maturity in *Octopus*. J. Exp. Biol. 36, 1–33.

Wooton, R.J., Allen, J.R.M., Cole, S.J., 1980. Effects of body weight and temperature on the maximum daily food consumption of *Gasterosteus aculeatus* (L.) and *Phoxinus phoxinus* (L.): selecting an appropriate model. J. Fish Biol. 17, 695–705.

Zar, J.H., 1996. Biostatistical Analysis, 4th edition. Prentice-Hall, Englewood Cliffs, NJ. 663 pp.