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**Experimental evaluation of the energy balance in *Octopus vulgaris*, fed *ad libitum* on a high-lipid diet**

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## Abstract

A complete energy balance equation was estimated for the common octopus *Octopus vulgaris* at a constant temperature of 20 °C, fed *ad libitum* on anchovy fillet (*Engraulis encrasicolus*). Energy used for growth and respiration or lost with faeces and excreted ammonia was estimated, along with total energy consumption through food, for six specimens of *O. vulgaris* (with masses between 114 and 662 g). The energy balance equation was estimated for the specimens at 10-day intervals. During each 10-day interval, food consumed, body mass increase and quantity of faeces voided were measured. The calorific values of octopus flesh, anchovy flesh and faeces were measured by bomb calorimetry. Oxygen consumption and ammonia excretion rates were monitored for each specimen during three 24-h experiments and daily oxygen consumption and ammonia excretion were estimated. It was found that 58% of the energy consumed was used for respiration. The amount of energy invested in somatic and gonadal growth represented 26% of the total energy budget. The energy discarded through faeces was 13% of consumed energy. The estimated Assimilation Efficiency (*AE*) values of *O. vulgaris* feeding on anchovy (80.9 – 90.7 %) were lower than the *AE* values estimated for other cephalopod species with different diets of lower lipid content such as crabs or mussels. Specific Growth Rates (SGR) ranged between 0.43 – 0.95 and were similar to those reported for other high-lipid diets (bogue, sardine) and lower than SGR values found for low-lipid, high-protein diets (squid, crab, natural diet). Ammonia excretion peak (6 hours after feeding) followed the one of oxygen consumption (1hour after feeding). The values of atomic oxygen-to-nitrogen (O:N) ratio indicated a protein-dominated metabolism for *O. vulgaris*.

## Introduction

The common octopus, *Octopus vulgaris*, is one of the most studied cephalopods. It is a coastal and sedentary species, living at a depth of 0 - 200 m with a decreasing abundance with depth (Guerra 1981). It was thought to have a world-wide distribution in tropical, subtropical and temperate waters (Atlantic, Indian Ocean, western Pacific), but recent studies support that *O. vulgaris*, in a strict sense, seems to be a species restricted to the Mediterranean and Eastern Atlantic coasts (Mangold 1998; Söller et al. 2000). Extensive research has been carried out on the biology, physiology and behavior of *O. vulgaris*; a review was presented by Mangold (1983). It has a significant commercial value and is an important target species for the fisheries of many countries. Its culture is becoming an area of interest due to the rapid growth and high food conversion ratio of octopuses (Mangold 1983); however, the paralarval culture of octopus remains a bottleneck (Villanueva 1995; Vaz-Pires et al. 2004).

Analyses of energy balance utilization in cephalopods are rare. Complete energy balance equations have been estimated for two tropical octopuses, *Octopus cyanea* and *Octopus maya*, (Van Heukelem 1976) and for the Antarctic octopus *Pareledone charcoti* (Daly and Peck 2000). Combining data from the literature, O'Dor and Wells (1987) constructed a partial energy balance for *O. vulgaris*, while Wells and Clarke (1996), in a thorough review of cephalopod energetics, estimated preliminary energy budgets for *O. vulgaris*, making several assumptions for the components of the energy balance equation. In the present study, a complete energy balance equation for *O. vulgaris* was estimated, based on experimental data. Anchovy was chosen as food for the octopuses as it has a low market value and is a potential food source for industrial octopus on-growing.

## Materials and Methods

The energy balance equation that represents the flow of energy through the octopus (Wells and Clarke 1996) is  $C = P + G + E_R + E_U + F$ , where  $C$  is the total energy content of food consumed,  $P$  and  $G$  are the energy equivalents of somatic and gonadal growth respectively,  $E_R$  is the energy utilized in respiration,  $E_U$  is the energy lost as nitrogenous and other waste compounds excreted in the urine, and  $F$  is the unabsorbed energy voided with the faeces.

Six octopuses, ranging in mass from 114 to 642 g, were collected by SCUBA and free diving in the Saronicos Gulf (37°30'N-37°55'N, 23°E-24°E). After collection, the animals were immediately placed into 40-l plastic holding tanks, with sufficient aeration provided by portable air pumps. Within 2 h they were transferred to the laboratory and each octopus was put in a separate 106 l holding tank; the ratio of holding tank volume to animal body mass was in every case more than 150 l kg<sup>-1</sup>. The tanks were connected to a 2-m<sup>3</sup> closed seawater system, filled with natural seawater. The system included a biological filter (300 l of smashed coral of ~2mm diameter; 5 l min<sup>-1</sup> filtration rate) for nitrification, a seaweed filter (culture of the green alga *Pseudochlorodesmis furcellata*) for nutrient accumulation, a sedimentation tank, a protein skimmer (Aqua Medic – Turboflotor 1000 multi) and a mechanical filter with a synthetic floss medium to remove suspended particles, and a 14-W UV lamp (Rena) for sterilization of the seawater. Water temperature was controlled by the combined continuous function of an air-conditioning device and a flow-through water-cooling device (AquaMedic, Titan 500). Detailed description of the closed seawater system is given by Katsanevakis (2004) and Katsanevakis et al. (2005a). Before the experiments, the octopuses were subjected to a one month acclimatization period, during which they adapted to their new environment and feeding on defrosted anchovy (*Engraulis encrasicolus*).

Throughout the experiment, temperature remained at 20 °C, salinity at 38.5 ± 0.2 psu and pH ranged between 7.8 and 8.1. There was a photoperiod of 12 h light – 12 h darkness, with light period between 0700 and 1900 hours. A plastic pot was placed in all holding tanks, to be used by the octopus as a den and the sides of the holding tanks were covered with a black self-adhesive surface, to avoid stress or excess activity, due to people moving around in the laboratory.

The energy balance equation was estimated for each octopus, during a 10-day interval. The specimens were weighed just before and after the 10-day intervals, without being anaesthetised, keeping the handling time as brief as possible to minimize stress. During the experimental period octopuses were fed *ad libitum* once a day (at 1300), with defrosted anchovy fillets (*E. encrasicolus*). One hour later the food remains were removed and weighed. The net wet mass of food consumed each day was recorded. 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 on the bottom or the sides of the tanks were removed by sucking through a tube, which ended at a 500 µm nylon net.

The oxygen consumption rate ( $R$ , in mg h<sup>-1</sup>) and ammonia excretion rate ( $U$ , in mg h<sup>-1</sup>) were monitored for each specimen, during three 24-hour experiments, in the 10-day interval. The experiments were conducted in the holding tanks and no separate respiration chamber was used, in order to avoid increased respiration and abnormal behaviour of the octopuses, due to stress caused by transportation. For each 24-hour experiment, 16 1-hour measurements of  $R$  and  $U$  were conducted, with 0.5-hour resting intervals between them. During the 1-hour measurement periods, the holding tank of the octopus being measured was isolated from the closed system. During the 0.5-hour

resting intervals the holding tank was reconnected to the closed system and with a combination of intense aeration with air-stones and recirculation of water (recirculation rate  $> 3 \text{ l min}^{-1}$ ) oxygen resaturation and sufficient ammonia depletion were achieved.

In each 1-hour interval, the dissolved oxygen concentration of 500-ml water samples, taken every 15 min, was measured with a WTW (Wissenschaftlich-Technische Werkstätten) polarographic oxygen probe (Cellox 325), which was connected to a WTW (MultiLine P4) meter, while the samples were stirred gently with a magnetic stirrer. After each oxygen measurement, the sample was returned to the holding tank undisturbed, with natural flow, through a 5-mm rubber tube.  $R$  was estimated from the slope of the linear regression, fitted to the oxygen concentration time series of each 1-hour interval. *O. vulgaris* is able to maintain a constant  $R$  regardless of the ambient dissolved oxygen concentration (metabolic regulator) provided that oxygen concentration is above a critical value, which is  $2.0 \text{ mg l}^{-1}$  according to Maginniss and Wells (1969) or  $2.3 \text{ mg l}^{-1}$  according to Valverde and Garcia (2005). To obtain a constant  $R$  in our measurements and a good linear fit to the oxygen-concentration time-series data, when oxygen concentration in the experimental tank fell below  $2.5 \text{ mg l}^{-1}$ , measurements were terminated earlier, i.e. before the end of the 1-h period.

Furthermore, three 50 ml water samples were taken at the beginning and three at the end of each 1-hour interval. The samples were frozen immediately. At the end of the 10-day trials, the ammonia concentration of the samples was measured, by the phenolhypochlorite method (Liddicoat et al. 1975) and the mean value of each triplet of samples was calculated.  $U$  was estimated by subtracting the initial from the final ammonia concentration of the holding tank, multiplied by the tank volume and divided by the time interval (1 h).

Thus, the diurnal variation of  $R$  and  $U$  was evaluated for each 24-hour experiment. Measurements of  $R$  and  $U$  in the same tanks with the same procedure but without animals (blanks) revealed that the rate of oxygen uptake or ammonia production due to microbial action or diffusion were not statistically different from zero and consequently ignored (for  $R$ :  $n = 15$ ,  $t = -2.02$ ,  $p=0.06$ , and for  $U$ :  $n = 12$ ,  $t = 0.62$ ,  $p=0.55$ ).

Total amounts of oxygen consumed and ammonia excreted in the 10-day period were estimated by numerical integration (using the trapezoid method) of the 10-day time-series of the oxygen consumption rate and ammonia excretion rate respectively.

The oxygen-to-nitrogen atomic ratio (O:N) was estimated for each specimen, using the formula  $62.5 \times R \times U^{-1}$ , where  $R$  is in  $\text{mg h}^{-1}$ ,  $U$  is in  $\mu\text{mol h}^{-1}$ , and 62.5 is a coefficient to convert  $\text{mg O}_2$  to  $\text{g-at O}$ . The time-series of the mean O:N ratio of all specimens was also estimated.

The octopus is an ammonotelic organism, with two thirds of nitrogen excreted in the form of ammonia, mainly through the gills; the rest is urea with a little uric acid (Andrews 1988; Hoeger et al 1987; Boucher-Rodoni and Mangold 1994). In the present study,  $E_U$  was estimated by the energy content of the ammonia excreted.

The energy content of octopus flesh, anchovy flesh and the faeces of each octopus were estimated by burning freeze-dried samples in a bomb calorimeter (IKA-Calorimetersystem C 4000 A). The mean calorific coefficients of five samples of octopus flesh, five samples of anchovy flesh and two samples from the collected faeces of each octopus were determined. The water content of octopus and anchovy flesh was measured by freeze drying appropriate samples and the wet-to-dry conversion factors were calculated.

The Specific Growth Rate ( $SGR$ ) and the % Assimilation Efficiency ( $AE$ ) of each specimen were estimated using respectively the equations

$SGR = 100(\ln W_f - \ln W_i)t^{-1}$ , where  $W_f$  and  $W_i$  are the final and initial mass respectively, in g, and  $t$  the time in days, and  $AE(\%) = 100(C - F)C^{-1}$ .

## Results

The mean food consumption rate, faeces production rate, mean  $R$ , mean  $U$ ,  $SGR$  and  $AE$  for each specimen, which all correspond to the 10-day experimental period, are given in Table 1.

For each specimen and each 24-hour experiment, both  $R$  and  $U$  values were standardized by dividing with the corresponding values in time 0 ( $R_0$  and  $U_0$  respectively). Time 0 refers to the period 1200 to 1300 hours, which represents the measurement just before feeding. Such standardization is necessary for comparing the oxygen consumption and ammonia excretion rates of different specimens. The mean values for the three 24-hour time-series of  $R/R_0$  and  $U/U_0$  respectively were calculated for each specimen. The mean values of  $R/R_0$  and  $U/U_0$  for all the specimens (for corresponding times) were then calculated (Fig. 1). The peak of the mean  $R/R_0$  was  $1.63 \pm 0.36$  (mean  $\pm$  standard deviation), in the first hour after feeding (1330 - 1430). The peak of the mean  $U/U_0$  was  $2.50 \pm 0.93$  at time  $t = 7.5$ h, approximately 6 hours after the  $R/R_0$  peak.

Mean O:N values varied from 7.2 to 15.6, with a mean value of  $10.0 \pm 2.4$  (Fig. 2). The O:N peak ( $15.6 \pm 4.8$ ) was observed in the second hour after feeding and then the O:N ratio declined sharply, remaining at low levels.

The components of the energy balance equation were estimated using the formulas of Table 2. The estimated components of the energy balance equation are given in Table 3 and refer to the 10-day experimental periods.

The energy balance for each specimen is demonstrated in Fig.3. The components are expressed as percentages of the calorific energy of food. Taking into account the mean values of the components, 58% of consumed energy was required for respiration. For somatic and gonadal growth, 26% of consumed energy was used, while 13% was voided through faeces.

## Discussion

Van Heukelem (1976) estimated that in young *Octopus cyanea* fed *ad libitum* on crabs, metabolic losses accounted for 35% of the energy of food consumed, while 57% of consumed energy went to growth and 3.5% to faeces. Daly and Peck (2000) estimated that in the Antarctic octopus *Paraleledone charcoti* fed on mussels at 0 °C, 68% of consumed energy went to fuelling metabolism, 29.8% to growth and 3.9% to faeces. In *O. vulgaris*, the relative energetic cost of respiration was higher than that for the tropical *O. cyanea* and lower than that for the polar octopus *P. charcoti*. These comparative data, albeit few, go against the general trend for cold water taxa to have higher growth efficiencies. It has to be noted, though, that (i) the comparison involves fast-growing young stages with slower-growing older stages, and (ii) each species has an optimum temperature for growth, which depends on body mass (Miliou et al 2005), and it might be the case that the three species compared were maintained at temperatures of different proximity to their optimum temperature.

The estimated  $AE$  values of *O. vulgaris* feeding on anchovy (80.9 – 90.7 %) were lower than the  $AE$  values estimated for other cephalopod species fed on different diets; three specimens of *P. charcoti* fed on mussels (*Mytilus edulis*) had  $AE$  between 95 and 97 % (Daly and Peck 2000) and *O. cyanea* fed on crabs had mean  $AE = 96.50$  % (Van Heukelem 1976). According to O'Dor and Wells (1987) cephalopods feeding on

high fat meals (e.g. sardines, anchovies etc.), produce faeces that float, indicating inefficient lipid absorption. General observations of digestive mechanisms support this idea (Vonk 1962), while other studies, have also shown that the capacity of cephalopods to metabolize lipids is limited (Mommensen and Hochachka 1981; O'Dor et al. 1984). Because of the high calorific value of lipids, a failure to assimilate them would cause a disproportionate effect on assimilation efficiency of cephalopods on high fat diets, even if they assimilate carbohydrate and protein with nearly 100% efficiency (O'Dor and Wells 1987). This could be a possible explanation for the relatively low mean *AE* value of this study, as anchovies that were used as food have high fat content in relation to mussels or crabs.

Octopuses cultured in the same closed-system but fed on squid (*Loligo vulgaris*) had higher SGR's (Miliou et al. 2005) than those of the present study (octopuses fed on anchovies); squid have approximately 6.4 % total lipid content on a dry matter basis (Silva and Chamul 2000). Higher growth rates than those of the present study were also reported for octopuses fed with crabs (Mangold 1983); crabs have a lipid content of 2.9-5.1 % according to Silva and Chamul (2000). The SGR levels estimated for *O. vulgaris* in its natural habitat, in the same geographical area as the one in which the specimens of the present study were collected, were also higher ( $1.6 \pm 0.30\% \text{ day}^{-1}$  for 50–200 g and  $1.19 \pm 0.31\% \text{ day}^{-1}$  for 200–500 g) (Katsanevakis and Verriopoulos in press). García and Giménez (2002) fed 24 *O. vulgaris*, ranging in mass from 259 to 487 g, on two different diets, bogue (*Boops boops*), with lipid content on a dry matter basis  $20.1 \pm 8.7\%$ , and sardine (*Sardina pilchardus*), which is a very fatty fish (lipid content on a dry matter basis was  $49.7 \pm 7.6\%$ ), at 17-21 °C. They found that bogue-fed individuals achieved significantly higher growth rates (SGR = 0.95) than those fed on sardine (SGR = 0.53). The SGR values of the present study, with anchovy-fed octopuses ranged between 0.43 and 0.95 and are similar to those of García and Giménez (2002); anchovy has a lipid content of approximately 18.4% according to Silva and Chamul (2000) which is close to the lipid content of bogue. The low SGR values in high-lipid diets (bogue, anchovy, sardine) in comparison to other diets (squid, crab, natural diet) and the low *AE* values of the present study support that, for octopuses, high-lipid diets are less efficient and have a reduced capacity for growth in comparison to low-lipid and high-protein diets.

Although ammonia is the main excretory product of octopuses (Andrews 1988; Hoeger et al 1987; Boucher-Rodoni and Mangold 1994), it is not the only nitrogenous compound excreted; in some octopus species urea may comprise as much as 30% of the nitrogen excretion (Hoeger et al 1987). Urine in the renal sacs contains substantial quantities of protein, and the corresponding energetic cost due to protein loss for a growing octopus may reach 2% of the total energy budget (Wells and Clarke 1996). In the present study, only ammonia excretion was measured and thus  $E_U$  was underestimated; nevertheless, such an error has little effect on the total energy budget.

The *R* peaked during the first hour after feeding which agrees with previous results for *O. vulgaris* fed on *E. encrasicolus* at 20 and 28 °C (Katsanevakis et al. 2005b) and also for *O. vulgaris* fed on crabs at 19-24 °C (Wells et al. 1983). The increase in *R* following food ingestion (specific dynamic action, SDA) is associated with the extra energy required for transportation of food down the alimentary tract, its digestion, absorption and post-metabolic processing (Jobling 1981). The increase of *U* after food intake is related to the assimilation of digestion products (predominately from protein metabolism). The peak of *U* (Fig. 1) was observed at  $t=7.5$  h, i.e. 6 hours after the one of *R*. The fact that *R* and *U* peaks follow one another and do not appear simultaneously has also been observed in other species (e.g. in the New Zealand rock

lobster *Jasus edwardsii*, Radford et al. 2004). SDA reflects the energy requirements of the behavioral, physiological and biochemical processes that constitute feeding including capture, handling, ingestion, digestion, and especially assimilation of prey and the increased synthesis of proteins and lipids associated with growth. The increase of  $R$  begins with the capture and handling of prey, while ammonia is the end-product of the degradation of the nitrogenous compounds of food, predominantly proteins and nucleic acids, and its excretion starts to increase some time after the capture and ingestion of food; thus the time lag between the  $R$  and  $U$  peaks is explainable.

The O:N ratio is widely used as a metabolic index. By theoretical computations it can be shown that pure metabolic protein catabolism will yield O:N ratios ranging from 3 to 16, while equal amounts of lipid and protein catabolism will correspond to values between 50 and 60 (Mayzaud and Conover 1988). The low O:N ratio found in our study, is indicative of a protein dominated catabolism, which is also supported by Katsanevakis et al. (2005a).

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**Table 1**

Experimental data for each of the six specimens of *Octopus vulgaris*. Values correspond to the whole 10-day interval.

	SPECIMEN					
	1	2	3	4	5	6
<b>INITIAL WET BODY MASS (g)</b>	114	642	523	350	318	414
<b>Food consumed (g of dry mass)</b>	4.45	19.62	23.87	9.79	8.32	14.18
<b>Somatic growth (g of dry mass)</b>	1.87	5.55	4.62	2.50	2.44	4.47
<b>Faeces (g of dry mass)</b>	0.68	2.98	5.86	2.74	1.80	2.25
<b>Calorific value of faeces <math>S_f</math> (J g<sup>-1</sup>)</b>	12282	12504	13459	13654	13919	14089
<b>Food consumption rate (g day<sup>-1</sup>)</b>	0.45	1.96	2.38	0.98	0.83	1.42
<b>Specific growth rate, SGR (%)</b>	0.95	0.52	0.53	0.43	0.45	0.64
<b>Mean oxygen consumption rate, <math>R</math> (mg h<sup>-1</sup>)</b>	13.0	67.1	69.6	36.7	31.7	66.7
<b>Mean ammonia excretion rate, <math>U</math> (μmole h<sup>-1</sup>)</b>	104	451	902	223	222	420
<b>Faeces production rate (mg dry mass day<sup>-1</sup>)</b>	67.5	298.0	585.8	274.3	180.0	225.0
<b>Assimilation efficiency, AE (%)</b>	90.7	90.5	83.5	80.9	85.0	88.8

**Table 2**

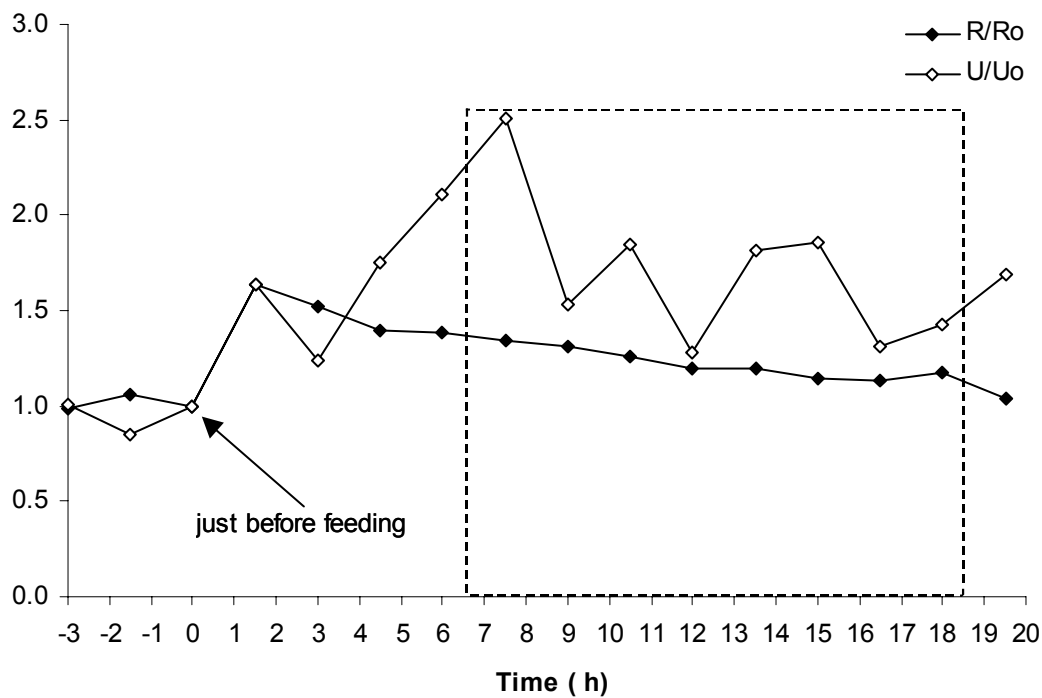
The formulas used for the estimation of the components of the energy balance equation. Subscript (s) denotes components experimentally estimated in the present study, while subscript (L) denotes literature values (the relevant citation is given in the ‘Remarks’ column).

<b>Components of energy balance equation</b>	<b>Remarks</b>
$C = 0.227_{(s)} \times 20019_{(s)} \text{ J g}^{-1} \times (\text{wet weight of consumed food})$	0.227: wet-to-dry mass conversion coefficient of food 20019 J g <sup>-1</sup> : calorific value of anchovy flesh (bomb calorimetry)
$E_R = 13.4_{(L)} \text{ J g}^{-1} \times (\text{consumed oxygen amount})$	13.4 J g <sup>-1</sup> : coefficient based on fuelling metabolism, using the relative percentage of protein, carbohydrate and lipid present in anchovy (Silva and Chamul 2000)
$E_U = 288_{(L)} \text{ J mol}^{-1} \times (\text{excreted ammonia amount})$	288 J mol <sup>-1</sup> : Brafield and Soloman 1972
$F = S_{f(s)} \times (\text{faeces dry weight})$	$S_f$ : calorific value of faeces (bomb calorimetry), see Table 1
$P + G = 0.162_{(s)} \times 17522_{(s)} \text{ J g}^{-1} \times (\text{wet weight of the body mass gained})$	0.162: wet-to-dry mass conv. coeff.of octopus flesh 17522 J g <sup>-1</sup> : calorific value of octopus flesh (bomb calorimetry)

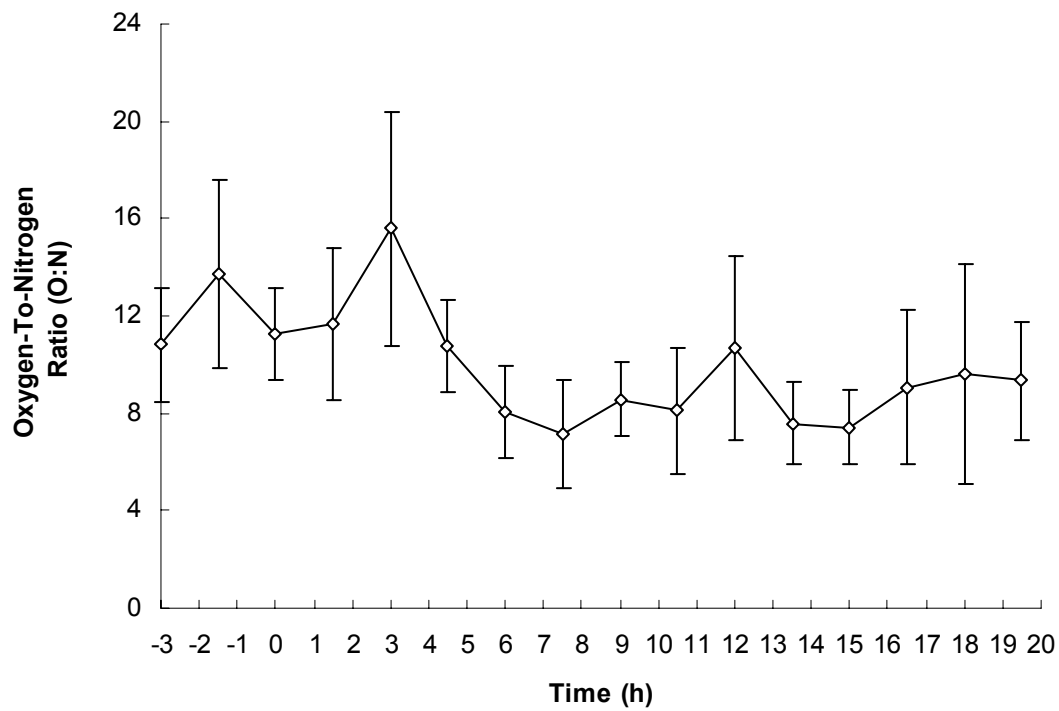
**Table 3**

Components of the energy balance equation for the six specimens of common octopus *Octopus vulgaris*. Values correspond to the whole 10-day interval.

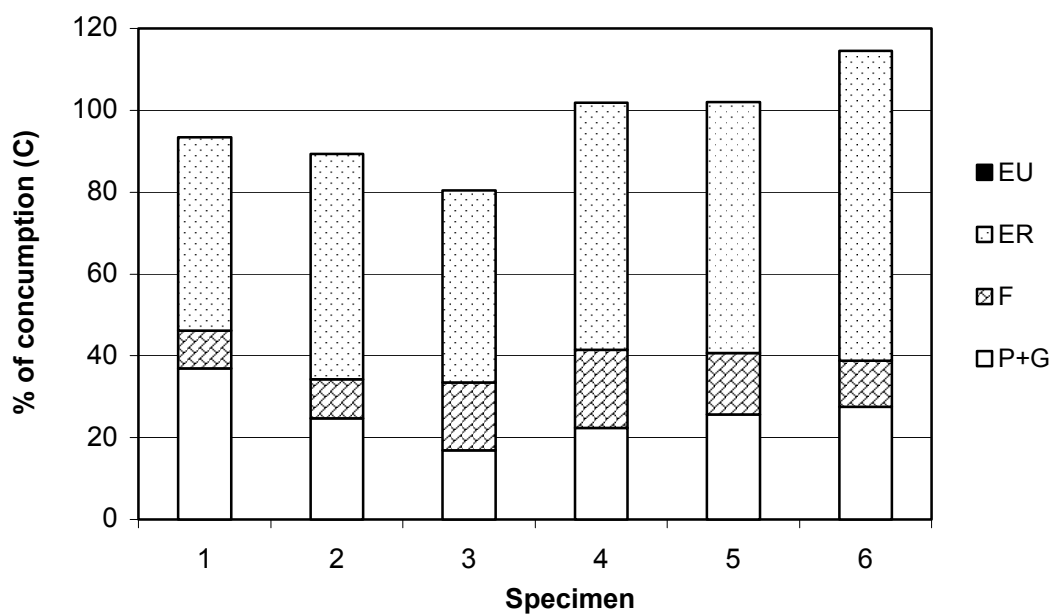
	SPECIMEN					
	1	2	3	4	5	6
$C$ ( $\text{J g}^{-1}$ )	88990	392737	477868	195914	166629	283769
$P+G$ ( $\text{J g}^{-1}$ )	32834	97224	80989	43778	42684	78253
$F$ ( $\text{J g}^{-1}$ )	8290	37262	78837	37457	25054	31700
$E_R$ ( $\text{J g}^{-1}$ )	42028	216352	224544	118251	102335	215126
$E_U$ ( $\text{J g}^{-1}$ )	7	31	62	15	15	29
$(P+G+F+E_R+E_U)/C$	<b>0,934</b>	<b>0,893</b>	<b>0,804</b>	<b>1,018</b>	<b>1,021</b>	<b>1,146</b>



**Fig. 1:** Diurnal variation of oxygen consumption ( $R/R_0$ ) and ammonia excretion ( $U/U_0$ ) rate of the common octopus *Octopus vulgaris*. Time 0 represents a 1h-interval before feeding (1200-1300 hours). The box represents darkness period.



**Fig. 2:** Oxygen-to-nitrogen (O:N) ratios variation of common *Octopus vulgaris* during a 24-hours period. Time 0 represents 1h-interval before feeding (1200-1300 hours). The error bars represent the standard deviation.



**Fig. 3:** Components of the energy budget for 6 specimens of the common octopus *Octopus vulgaris*, expressed as a percentage (%) of the calorific content of food consumed.