RESEARCH ARTICLE

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

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 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 h after

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feeding) followed the one of oxygen consumption (1 h 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 behaviour 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 ongrowing.

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^{-1}. The tanks were connected to a 2-m^3 closed seawater system, filled with natural seawater. The system included a biological filter (300 l of smashed coral of ∼2 mm diameter; 5 l min^{-1} 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 (Aqua-Medic, 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 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 h. 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^{-1}) and ammonia excretion rate (U, in mg h^{-1}) were monitored for each specimen, during three 24-h 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-h experiment, sixteen 1-h measurements of R and U were conducted, with 0.5-h resting intervals between them. During the 1-h measurement periods, the holding tank of the octopus being measured was isolated from the closed system. During the 0.5-h 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 l min^{-1}) oxygen resaturation and sufficient ammonia depletion were achieved.

In each 1-h interval, the dissolved oxygen concentration of 500-ml water samples, taken every 15 min, was measured with a Wissenschaftlich-Technische Werkstatten (WTW) 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-h 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 mg l^{-1} according to Maginniss and Wells (1969) or 2.3 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 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-h 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-h 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 mg h^{-1}, U is in μmol h^{-1}, and 62.5 is a coefficient to convert mg O_2 to 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 $\mathrm{SGR} = 100(\ln W_f - \ln W_i)t^{-1}$, where W_f and W_i are the final and initial mass respectively, in g, and t the time in days, and $\mathrm{AE}(\%) = 100(C - F)C^{-1}$.

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-h 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-h 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 ± 0.36 (mean ± standard deviation), in the first hour after feeding (1330–1430). The peak of the mean U/U_0 was 2.50 ± 0.93 at time $t = 7.5$ h, approximately 6 h after the R/R_0 peak.

Mean O:N values varied from 7.2 to 15.6, with a mean value of 10.0 ± 2.4 (Fig. 2). The O:N peak (15.6 ± 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 *O. 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 *Paraledone 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 (1) the comparison involves fast-growing young stages with slower-growing older stages, and (2) 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

**Table 1** Experimental data for each of the six specimens of *Octopus vulgaris*

|	Specimen					
	1	2	3	4	5	6
Initial wet body mass (g)	114	642	523	350	318	414
Food consumed (g of dry mass)	4.45	19.62	23.87	9.79	8.32	14.18
Somatic growth (g of dry mass)	1.87	5.55	4.62	2.50	2.44	4.47
Faeces (g of dry mass)	0.68	2.98	5.86	2.74	1.80	2.25
Calorific value of faeces S_f (J g^{-1})	12,282	12,504	13,459	13,654	13,919	14,089
Food consumption rate (g day^{-1})	0.45	1.96	2.38	0.98	0.83	1.42
Specific growth rate, SGR (%)	0.95	0.52	0.53	0.43	0.45	0.64
Mean oxygen consumption rate, R (mg h^{-1})	13.0	67.1	69.6	36.7	31.7	66.7
Mean ammonia excretion rate, U (µmol h^{-1})	104	451	902	223	222	420
Faeces production rate (mg dry mass day^{-1})	67.5	298.0	585.8	274.3	180.0	225.0
Assimilation efficiency, AE (%)	90.7	90.5	83.5	80.9	85.0	88.8

Values correspond to the whole 10-day interval

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 (Mommsen 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 AE 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 SGRs (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 ± 0.30% day^{-1} for 50–200 g individuals and 1.19 ± 0.31% day^{-1} for 200–500 g individuals) (Katsanevakis and Verriopoulos 2005). 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 ± 8.7%, and sardine (*Sardina pilchardus*), which is a very fatty fish (lipid content on a dry matter basis was 49.7 ± 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
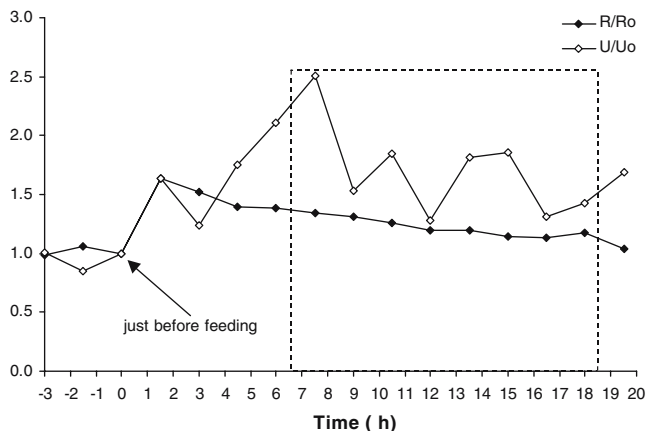
Fig. 1 Diurnal variation of oxygen consumption (R/R_0) and ammonia excretion (U/U_0) rate of *O. vulgaris*. Time 0 represents 1 h-interval before feeding (1200–1300 h). The *box* represents darkness period
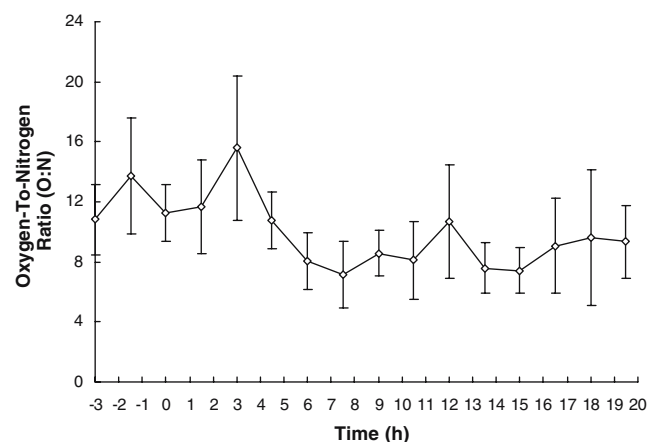
Fig. 2 Oxygen-to-nitrogen (O:N) ratios variation of *O. vulgaris* during a 24-h period. Time 0 represents 1 h-interval before feeding (1200–1300 hours). The *error bars* represent the standard deviation

Table 2 The formulas used for the estimation of the components of the energy balance equation

Components of energy balance equation	Remarks
$C = 0.227_{(s)} \times 20{,}019_{(s)}$ J g^{-1}×(wet weight of consumed food)	0.227: wet-to-dry mass conversion coefficient of food 20,019 J g^{-1}: calorific value of anchovy flesh (bomb calorimetry)
$E_R = 13.4_{(L)}$ J g^{-1}×(consumed oxygen amount)	13.4 J g^{-1}: coefficient based on fuelling metabolism, using the relative percentage of protein, carbohydrate and lipid present in anchovy (Silva and Chamul 2000)
$E_U = 288_{(L)}$ J mol^{-1}×(excreted ammonia amount)	288 J mol^{-1}: Brafield and Soloman (1972)
$F = S_{f(s)}$×(faeces dry weight)	S_f: calorific value of faeces (bomb calorimetry), see Table 1
$P + G = 0.162_{(s)} \times 17{,}522_{(s)}$ J g^{-1}×(wet weight of the body mass gained)	0.162: wet-to-dry mass conv. coeff. of octopus flesh 17,522 J g^{-1}: calorific value of octopus flesh (bomb calorimetry)

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)

Table 3 Components of the energy balance equation for the six specimens of octopus

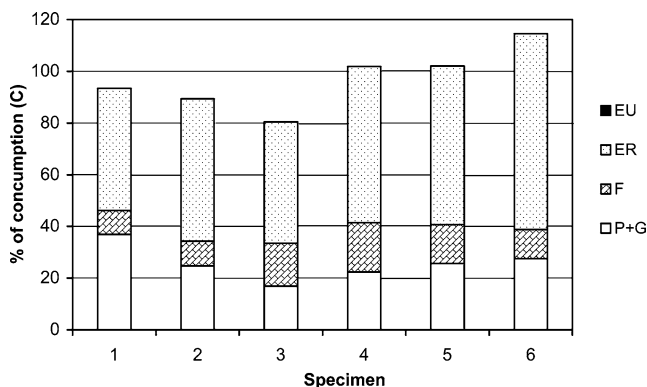|	Specimen					
	1	2	3	4	5	6
C (J g^{-1})	88,990	392,737	477,868	195,914	166,629	283,769
$P + G$ (J g^{-1})	32,834	97,224	80,989	43,778	42,684	78,253
F (J g^{-1})	8,290	37,262	78,837	37,457	25,054	31,700
E_R (J g^{-1})	42,028	216,352	224,544	118,251	102,335	215,126
E_U (J g^{-1})	7	31	62	15	15	29
$(P + G + F + E_R + E_U)/C$	0.934	0.893	0.804	1.018	1.021	1.146

Values correspond to the whole 10-day interval

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 h 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 behavioural, 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

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

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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