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Oxygen consumption and ammonia excretion of *Octopus vulgaris* (Cephalopoda) in relation to body mass and temperature

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Abstract

The common octopus, *Octopus vulgaris* Cuvier, is of great scientific and commercial importance and its culture is becoming an area of increasing interest. In this study, the combined effects of temperature (*T*) and body mass (*M*) on the routine oxygen consumption rate (*R*) and ammonia excretion rate (*U*) in *O. vulgaris* were quantified. The experiments were conducted in a closed seawater system, and great care was taken to reduce handling stress of the animals. Temperature, salinity, pH and ammonia, nitrite, nitrate and phosphate concentrations were monitored and controlled during the experiment. The following predictive equations were evaluated: $R(\mu mol/hr) = \mathrm{e}^{25.24-6952.8/T_a} \cdot M^{0.901}$ at temperatures between 13-28°C and $U(\mu mol/hr) = e^{14.77-4324.7/T_a} \cdot M^{0.896}$ at temperatures between 15.5-26°C (T_a is degrees Kelvin and *M* in gram). O/N ratios showed that *O. vulgaris* has a protein-dominated metabolism. No significant relationship between the O/N ratio and body mass or temperature was found. Sex had no significant effect on the oxygen consumption rate or on the ammonia excretion rate. For other octopod species, the dependence of metabolic rate on temperature does not differ with that for *O. vulgaris*.

Introduction

The common octopus, *Octopus vulgaris*, is the type species of the genus and one of the most studied cephalopods. It is of great scientific importance and has been used extensively for central nervous system investigations (Young 1964). Extensive research has been carried out on the biology, physiology and behavior of *O. vulgaris* (Mangold 1983). The common octopus has a significant commercial value and is an important target species for the fisheries of many countries. The culture of *O. vulgaris* is becoming an important 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).

The oxygen consumption (*R*) and ammonia excretion (*U*) rates of *O. vulgaris* have been studied thoroughly. Wells et al. (1983a) found that feeding and movement both lead to large increases in oxygen consumption and that nocturnal activity peaks represent major periods of energy expenditure. Wells et al. (1983b) determined the metabolic cost of walking for octopuses, which is about three times the cost of swimming for a fish of similar size and one-third the cost of tetrapod walking on land; maximum oxygen uptake during crawling was about 2.4 times their resting uptake. Specific dynamic action (SDA) peak levels and duration for an individual *O. vulgaris* at a constant temperature have been reported by Wells et al. (1983c). Wells and Wells (1985, 1995) indicated that *O. vulgaris* could regulate its oxygen uptake down to a P_{O2} of around 6.7kPa; as oxygen concentration decreases, octopuses regulate their oxygen uptake mainly by increasing their ventilation stroke volume. Boucher-Rodoni and Mangold (1985) found that in *O. vulgaris*, starvation induces a decline in *R* and *U* and an increase in the atomic O/N ratio, suggesting that *O. vulgaris* uses up other reserves (lipids) before utilizing an exclusively protein substrate. Madan and Wells (1996) showed that approximately 41% of the total oxygen requirements of an octopus at rest might be satisfied through cutaneous respiration. Parra et al. (2000) measured respiration rates in late eggs and early hatchlings of *O. vulgaris.* They found that oxygen consumption rates increase suddenly at hatching (approximately three times),

in response to the increase in energy expenditure due to the constant swimming activity of paralarva.

Among the exogenous and endogenous factors that influence oxygen consumption and ammonia excretion rates, temperature and mass are very important (Rao and Bullock 1954; Bertalanffy 1957; Kinne 1970; Gillooly et al. 2001). The effects of temperature on *R* and *U* for the complete temperature range in which the species is encountered and the combined effects of mass and temperature have not been studied. The purpose of the present study was to evaluate the combined effects of temperature and body mass on the routine *R* and *U* in *O. vulgaris*, defined as those measured during uncontrolled but minimal activity.

Materials and methods

Octopuses were collected by SCUBA and free diving in the Saronicos Gulf ($37^{o}30'$N-$37^{o}55'$N, 23^{o}E-24^{o}E) from April 2000 to June 2003. Immediately after collection the animals were placed into 40-L plastic holding tanks, equipped with portable air pumps, and within 2h were transferred to the laboratory. Experiments were performed at 6 test temperatures (13 ^{o}C, 15.5 ^{o}C, 20 ^{o}C, 25 ^{o}C, 26 ^{o}C and 28^{o}C), which cover the local range of coastal sea temperatures in the Saronicos Gulf (~13^{o}C in winter and up to 28^{o}C in summer). The incubation temperature did not differ by >2^{o}C from the sea-temperature, to avoid thermal stress and long acclimation times. Thus, the experiments at the low temperatures (13 and 15^{o}C) were conducted during winter months, the experiments at the high temperatures (25, 26 and 28^{o}C) during summer and early autumn and the 20^{o}C series during spring or late autumn. At all temperatures, juveniles, immature and mature animals were measured, according to the classification of Mangold-Wirz (1963), except at low temperatures (13 and 15 ^{o}C), when no juveniles were found. No females near spawning, spent females, or animals that did not feed regularly were measured. Before experiments, the octopuses were given an acclimatization period of 1 week in which to get used to their new environment and to feeding on frozen squid. A photoperiod of 12 h light – 12 h darkness was maintained; the light period was between 0800 and 2000 hours.

A 2-m^3 closed system was used, filled with natural seawater of 38.5$^{o}/_{oo}$ salinity. The seawater was circulated through 300L of smashed coral (appr. 2mm diameter), at a rate of 5 L/min, for nitrification. A culture of the green alga *Pseudochlorodesmis furcellata* was maintained (seaweed filter), in a 150x45x24cm glass tank with 8 cool-white 40 W lamps and a photoperiod of 16 h light – 8 h darkness. The seawater of the closed system was circulated through the *P. furcellata* culture in order to further reduce NH_3 and NO_2^- and to control the NO_3^- level. Seaweed filters have been used frequently in closed systems as nutrient reductors (Neori et al. 1996; Neori et al. 1998; Jones et al. 2001), however *P. furcellata* has not previously been reported for such an application. A protein skimmer (Aqua Medic – Turboflotor 1000 multi) was used to remove suspended particles, and a 14W UV lamp (Rena) was used for sterilization of the seawater. Biological filter effluents flowed into the sedimentation tank, which served as a settlement tank for suspended particles and as a reservoir of relatively increased dynamic energy seawater; this water was used to supply all the holding tanks with a constant natural flow of recycled seawater. The bottom of the sedimentation tank was cleaned weekly. The inflow of each holding tank was regulated with a spherical valve. Ten holding tanks were connected to the closed system with the following capacities: 3x106 l, 3x18.6 l, 2x21 l and 2x13

l. The outflow of the holding tanks was directed, through a mechanical filter with a synthetic floss medium, to the biological filter. Water temperature was controlled by the combined continuous function of an air-conditioning device and a flow-through water-cooling device (AquaMedic, Titan 500).

Salinity and pH were monitored daily. Increases in salinity due to evaporation were corrected with the addition of deionized water and salinity remained at 38.5±0.4$^{o}/_{oo}$ throughout the experiment. There was a trend towards a decline in pH, mainly due to respiration and nitrification; this trend was corrected regularly with the addition of sodium bicarbonate (Spotte 1992). pH ranged between 7.7 and 8.1 during the experiment. Ammonia, nitrite, nitrate and phosphate concentrations were monitored twice weekly. In the central tank, mean and maximum values, respectively, of ammonia were 2.9 and 6.5 μmol l^{-1}, of nitrite 1.2 and 2.9 μmol l^{-1}, of nitrate 5.1 and 7.4 mmol l^{-1} and of phosphate 0.17 and 0.25 mmol l^{-1}.

To minimize stress, a plastic pot was placed in all holding tanks to be used by the octopus as a den, and the ratio of holding tank volume to animal weight was in every case more than 50 l kg^{-1}. The experiments were conducted in the holding tanks, and no separate respiration chamber was used so that the octopuses were well adapted to their artificial environment and no stress due to transportation to a new chamber was caused. The sides of the holding tanks were covered with a black self-adhesive blind, to avoid stress due to people moving around in the laboratory. Given that diurnal changes in activity and metabolic rate that are affected by feeding have been observed in *Octopus vulgaris* (Wells et al. 1983a), the timing of measurements and feeding was kept constant. Measurements were conducted in the morning (between 9:00 and 13:00) and feeding time was always between 14:00 and 15:00. Feeding has not only a short-term effect on metabolic rates (specific dynamic action), but also affects the standard metabolism of octopuses (Wells et al. 1983c). Furthermore, feeding ration affects the activity pattern of *O. vulgaris* during the following hours (Wells et al. 1983a) and, thus, the *R* and *U* values. To reduce the possible effect of differing daily rations on the *R* and *U* measurements, octopuses were always fed with a quantity of food equal to 3% of their net weight. That amount of daily ration was sufficient to induce growth at all temperatures, and it was fully consumed in most cases. Whenever octopuses did not consume the entire ration given on the previous day (more often at lower temperatures), they were excluded from the experiment.

During every measurement, the holding tank of the octopus measured was isolated from the closed system by closing the inflow valve, aeration was switched off, and the tanks were completely filled with seawater so that no air remained inside. Through two small holes (10 mm diameter) at the cover of the tank, two 5-mm rubber tubes were inserted with their ends at different positions inside the experimental tanks and remained there throughout the experiment. Two 500-ml water samples were drained with natural flow via the two 5-mm rubber tubes from the experimental tank every 15 min, for a period of 2.5 h. Dissolved oxygen for the samples was measured with a WTW (Wissenschaftlich-Technische Werkstatten) polarographic oxygen probe (Cellox 325) connected to a WTW (MultiLine P4) meter, while stirring the samples gently with a magnetic stirrer. In most cases, there was no gradient in oxygen concentration inside the tank, as the respiratory mechanism of the octopus proved to be a sufficient circulating device. However, when the oxygen concentration of the two samples differed by >0.2 mg l^{-1}, a plastic stick was inserted through one of the 10-mm holes at the cover of the tank and used to mix the water carefully; except for the two 10-mm holes there was no air-water interface, and thus the amount of air introduced by mixing with the plastic stick was negligible. The mean of the two measurements

was taken as the oxygen concentration of the experimental tank. After each oxygen measurement, the water of the samples was returned to the holding tank with natural flow via the 5 mm rubber tubes. If the oxygen concentration in the experimental tank fell below 2.5 mg l^{-1}, the experiment was terminated earlier, since below 2 mg l^{-1} octopuses begin to show signs of distress and oxygen consumption declines (Maginniss and Wells 1969). At the beginning of the experiment (time zero) and at the end (after 2.5 h), three 50-ml samples were taken on each sampling occasion. The ammonia concentration of the samples was measured using the phenolhypochlorite method (Liddicoat et al. 1975), and the mean value was calculated. A holding tank of the same volume, but with no octopus, was used as a control, and exactly the same procedure was followed.

A time series (from 0 to 2.5 h) of the oxygen content of the tank was calculated from the product of the oxygen concentration measurements and the tank volume. The declining rate of oxygen content was calculated as the slope of the least squares line that fitted the data. The same was done for the control, and the oxygen consumption rate (R) of the octopus was calculated as the difference between the two slopes (experimental tank-control). In this way, the potential contribution of bacterial respiration was taken into account. The control correction was usually less than 1%, although in some cases (at high temperatures) it reached 9.5%. The ammonia excretion rate (U) was calculated from the difference of the two ammonia concentration measurements at the beginning and at the end of the 2.5-h interval, after control correction. For each octopus, measurements of R and U were performed three times on three successive days, and the mean of the three replicate values was taken as the specimen's R or U respectively. In this way, a total of 108 R-values were estimated at the six test temperatures using animals with body masses between 19 and 2160g. A total of 68 U-values were estimated at four test temperatures (15.5 ^{o}C, 20 ^{o}C, 25 ^{o}C, 26^{o}C) using specimens of a body mass between 19 and 1210 g. The body mass of each octopus was measured twice, 2 days before the start of the three-day measurement period and on the third day, immediately after the measurement and before feeding. The body mass of the octopus on each measurement day was calculated by linear interpolation between the two body mass values. The reason for measuring the body mass two days before the start of the measurements was to give the specimens enough time to overcome the handling stress of weighting; a 24-h period is enough for octopuses to recover from handling stress (Wells et al. 1983a; author's personal observations), and the maximum recovery time recorded for fish was 48 h (Papoutsoglou et al. 1999). Octopuses were removed from their tanks using nets, quickly placed in a plastic container, weighted and then returned to their tanks. The water released from their mantle cavity remained in the plastic container and was weighted and subtracted from total mass. The specimens were not anaesthetized, and handling time was kept as brief as possible.

The dependence of a biological variable Y (which in our case is R or U) on body mass M is typically characterized by an allometric scaling law of the form $Y=Y_0M^b$, where b is the scaling exponent and Y_0 a constant that is characteristic of the kind of organism (West et al. 1997). To estimate the scaling exponent for the oxygen consumption rate ($Y=R$) and for the ammonia excretion rate ($Y=U$) of *O. vulgaris*, least squares linear regressions were calculated between the log-transformed R (mg h^{-1}) or U (μmol h^{-1}) *versus* log transformed body mass (M, in g) at each temperature. The general equations were of the form (Zeuthen 1953; Bertalanffy 1957):

$$\log(R \text{ or } U) = a + b \cdot \log M \qquad \text{(Eq.1)}$$

To confirm that b is independent of temperature, the slopes of the regression equations between log$R$ or log$U$ and body mass were compared with ANOVA at the various temperature levels. The common slope was the scaling exponent b (b_R for R and b_U for U). Each R or U measurement was scaled by dividing with M^b, and the mass-normalized oxygen consumption rate and ammonia excretion rate were $R_0 = R/M^{b_R}$ and $U_0 = U/M^{b_U}$ respectively. Temperature governs metabolism through its effects on rates of biochemical reactions, and this dependence is well approximated with the relationship (Gillooly et al. 2001):

$$R_0 \text{ (or } U_0) = c \cdot \mathrm{e}^{-E_i/kT_a} \Leftrightarrow \log(R_0 \text{ or } U_0) = \log c - \frac{E_i}{k} \cdot \frac{1}{T_a} \qquad \text{(Eq.2)}$$

where E_i is the activation energy, $k = 8.618 \cdot 10^{-5}$ eV K^{-1} is Boltzmann's constant and T_a is the absolute temperature (in degrees Kelvin). To estimate the parameters of Eq.2, least squares linear regressions were calculated between logR_0 (or logU_0) and $1/T_a$.

The temperature dependence of R (or U) is given by the 'universal temperature dependence' (UTD):

$$UTD = \exp(E_i T_c / kTT_0) = \exp\left\{E_i T_c / kT_0^2 (1 + T_c / T_0)\right\} \qquad \text{(Eq.3)}$$

where temperatures are in degrees Kelvin, T_0 is a reference temperature and $T_c = T - T_0$ (Gillooly et al. 2001). Eq.3 also expresses the temperature dependence in terms of degrees Celsius by choosing T_0=273.1K (the freezing point of water), in which case T_c defines temperature in degrees Celsius. Thus, having estimated E_i by the linear regression of Eq.2, UTD is calculated from Eq.3, as a function of temperature (in ^{o}C). Although UTD has advantages over the traditional Q_{10} factor (Gillooly et al. 2001), Q_{10} is widely used; thus, it was also calculated so that our results may be compared with other studies. Q_{10} between a temperature T_c (in ^{o}C) and T_0=273.1K, can be calculated with the equation (Gillooly et al. 2001):

$$Q_{10} = \exp\left(10 E_i / \left\{kT_0^2 (1 + T_c / T_0)\right\}\right) \qquad \text{(Eq.4)}$$

To check whether sex has a significant effect on R or U, two general linear models (GLM) were calculated (Glantz and Slinker 2001) with log-transformed R or U values as a dependent variable, log-transformed body mass as an independent quantitative variable and temperature and sex as independent categorical variables. Marginal (type III) sum of squares was used (Glantz and Slinker 2001).

The O/N ratio was calculated as the quotient R(μmol h^{-1})/U(μmol h^{-1}) for each individual separately, and then a multiple regression was estimated with the O/N ratio as the dependent variable and T and M as independent variables to investigate a possible dependence of O/N on T or M.

Results

The results of the oxygen consumption rate and ammonia excretion rate measurements for *Octopus vulgaris* in relation to body mass and temperature are presented in Fig. 1 and Fig. 2 respectively. The linear regression equations for log$R$ and log$U$ (Eq.1) are given in Table 1, and all of them are highly significant ($p<0.0001$). There were no statistical differences among the slopes of the log$R$ regressions ($p=0.56$) or among the slopes of the log$U$ regressions ($p=0.35$). On the contrary, the intercepts differed significantly, both among the log$R$ regressions ($p<0.0001$) and among the log$U$ regressions ($p=0.0001$). For log$R$ *versus* log$M$, the common slope (± standard error) was b_R=0.901±0.019 and for log$U$ *versus* log$M$, it

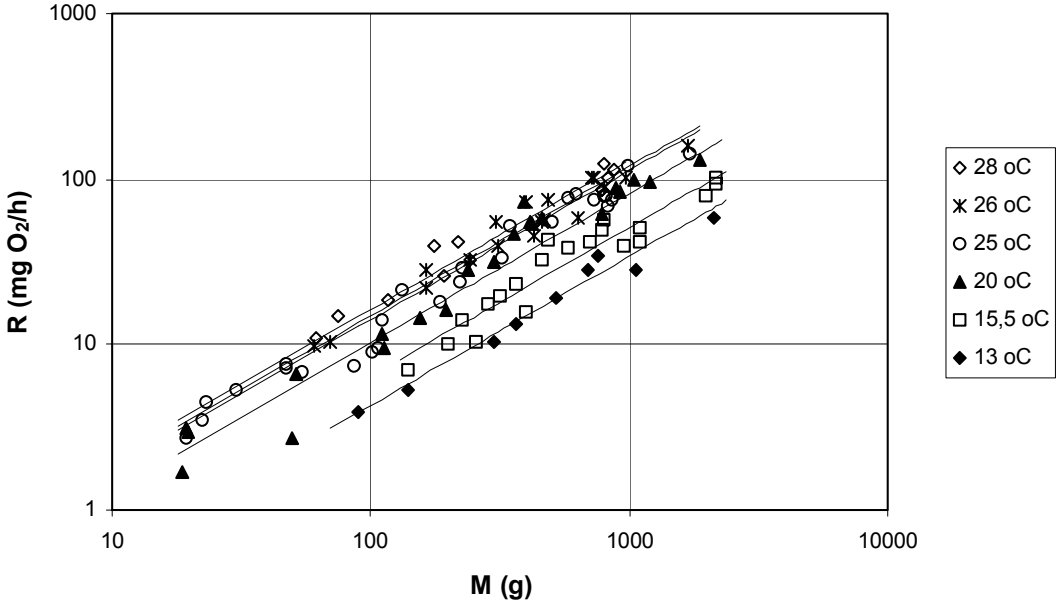

Fig. 1: *Octopus vulgaris*. Results of the oxygen consumption rate (*R*) measurements in relation to body mass (*M*) and temperature (*T*). Each point on the graph is the mean value of three measurements of a specimen, on consecutive days. The lines are the regression equations, calculated after forcing for equal slope (b_R=0.901).

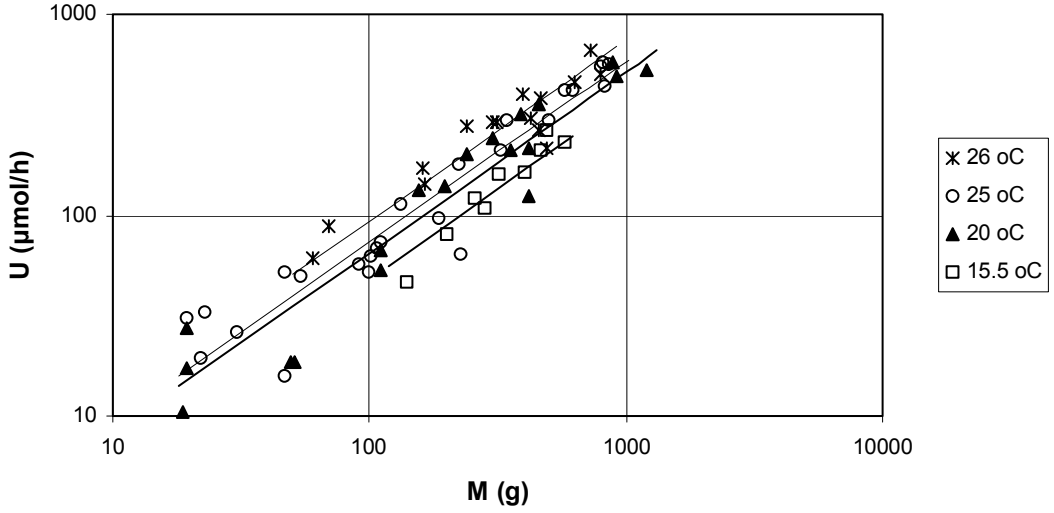

Fig. 2: *Octopus vulgaris.* Results of the ammonia excretion rate (*U*) measurements in relation to body mass (*M*) and temperature (*T*). Each point on the graph is the mean value of three measurements of a specimen, on consecutive days. The lines are the regression equations, calculated after forcing for equal slope (b_U=0.896).

was b_U=0.896±0.036; the corresponding (common slope) regressions are presented in Figs 1 and 2.

The linear regression equations for logR_0 and logU_0 (Eq.2) were respectively:

$$\log(R_0) = 21.80 - 6952.8 \cdot {}^{1}\!/\!_{T_a} \qquad \text{(Eq.5)}$$

$$\log(U_0) = 14.77 - 4324.7 \cdot {}^{1}\!/\!_{T_a} \qquad \text{(Eq.6)}$$

and were both highly significant ($p<0.0001$). The 95% confidence intervals for the slopes were, respectively, 6952.8±819.5 K and 4324.7±1852.4 K.
From Eq.2, Eq.5 and Eq.6, we estimate the 95% confidence intervals of E_i:

$$\frac{E_{i(R)}}{k} = 6952.8 \pm 819.5\text{K} \Rightarrow E_{i(R)} = 8.618 \cdot 10^{-5}\ \text{eV/K} \cdot (6952.8 \pm 819.5)\text{K} = 0.599 \pm 0.071\text{eV} \quad \text{(Eq.7)}$$

$$\frac{E_{i(U)}}{k} = 4324.7 \pm 1852.4\text{K} \Rightarrow E_{i(U)} = 8.618 \cdot 10^{-5}\ \text{eV/K} \cdot (4324.7 \pm 1852.4)\text{K} = 0.373 \pm 0.160\text{eV} \quad \text{(Eq.8)}$$

Thus, the combined effects of body size and temperature on R and U are given by the following models, respectively:

$$R(mg/hr) = R_0 \cdot M^b = \text{e}^{21.80 - 6952.8/T_a} \cdot M^{0.901} \Leftrightarrow$$
$$R(\mu mol/hr) = 31.25 \cdot \text{e}^{21.80 - 6952.8/T_a} \cdot M^{0.901} = \text{e}^{25.24 - 6952.8/T_a} \cdot M^{0.901} \quad \text{(Eq.9)}$$

$$U(\mu mol/hr) = U_0 \cdot M^b = e^{14.77 - 4324.7/T_a} \cdot M^{0.896} \quad \text{(Eq.10)}$$

where M is in grams and T_a is the absolute temperature. Eq.9 is valid in the temperature range 13-28°C (286.1-301.1K), while Eq.10 is valid in the range 15.5-26°C (288.6-299.1K).

UTD was calculated from Eq.3 and Q_{10} from Eq.4, for both R and U, and the results are given graphically in Fig. 3.

Sex was not statistically significant for either R ($p=0.75$) or U ($p=0.86$); thus, there is no dependence of the metabolic rates on sex.

The regression of the O/N ratio with T and M as independent variables was not significant ($df=(2, 63)$, $F=0.42$, $p=0.66$, $R^2=1.3\%$), and there was no significant dependence of the O/N ratio on either M ($df=63$, $t=-0.23$, $p=0.82$) or T ($df=63$, $t=0.89$, $p=0.38$). Thus, O/N was considered constant and independent of temperature and body mass and was calculated as the mean of all O/N values. O/N values ranged between 3 and 15 (mean ± standard deviation = 5.5 ± 2.4).

Table 1

Regression equations of *R* (mg/h) and *U* (µmol/h) *versus* body mass *M* (g) at different temperature levels.

Temperature (°C)	Regression equation	n	R^2
13	log*R* = -2.66 + 0.893 logM	9	0.97
15.5	log*R* = -2,33 + 0.907 logM	20	0.91
20	log*R* = -1.99 + 0.951 logM	22	0.96
25	log*R* = -1.53 + 0.886 logM	27	0.97
26	log*R* = -1.18 + 0.858 logM	18	0.95
28	log*R* = -0.92 + 0.829 logM	12	0.96
15,5	log*U* = -1.87 + 1.177 logM	9	0.95
20	log*U* = -0.16 + 0.937 logM	19	0.92
25	log*U* = 0.27 + 0.877 logM	26	0.91
26	log*U* = 1.16 + 0.768 logM	14	0.88

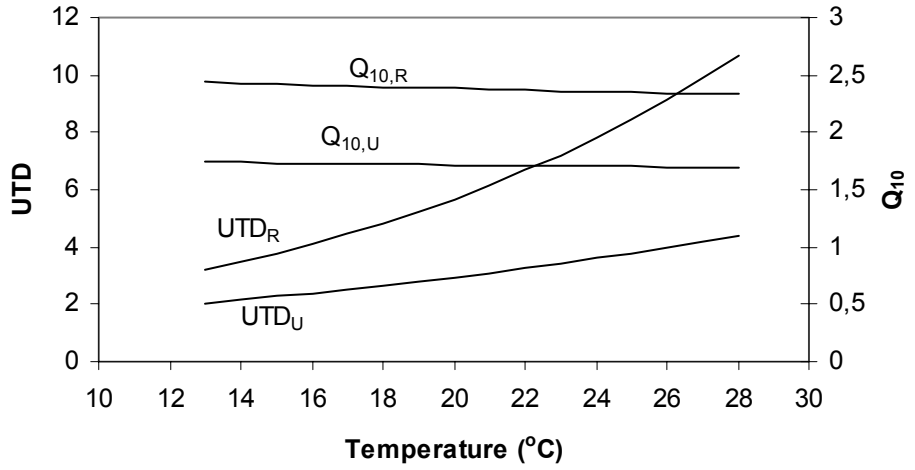

Fig. 3: ***Octopus vulgaris.*** **UTD and Q_{10} for oxygen consumption rates (*R*) and ammonia excretion rates (*U*), as functions of temperature.**

Discussion

Estimating *R* or *U*, through short-term experiments employing small respiratory chambers, may lead to abnormal responses, especially in a 'higher' organism (Kinne 1970) such as an octopus. Long-term assessments under experimental conditions, which allow normal growth rates or even normal reproductive activities, are to be preferred in such studies (Kinne 1970). Great efforts were made to apply an experimental procedure that minimizes handling stress and provides the octopuses with an environment as similar as possible to the natural one. The octopuses had positive growth rates, between 0.5 and 2.0% increase in body mass per day. Octopuses mated when placed together in a single tank, and spawning of eggs and hatching of paralarva occurred four times in the closed system used in this study, indicating good experimental conditions. The temperature range of our experiments covers the temperature range in which the species is mostly encountered, and all octopuses were acclimated to the experimental temperatures in every case. Thus, the predictive models of this study (Eq. 9 and 10) adequately estimate the standard metabolic rates of *Octopus vulgaris* in their natural environment at different seasons.

Boucher-Rodoni and Mangold (1985) measured *R* and *U* for two individuals of *O. vulgaris*, subjected to two feeding regimes, fed and starved, and at temperatures ~15.5°C. While the ammonia excretion rates estimated in this study are similar to those of Boucher-Rodoni and Mangold, their oxygen consumption rate values are about 3 times higher than ours. Wells et al. (1983a) estimated the oxygen consumption rate of *O. vulgaris* measuring 48 individuals with an average weight of 382±136g at a mean temperature of 21.3°C. For this weight range, the absolute difference of their values compared to ours is less than 7.5%; thus, the *R* estimated values of the two studies are in good agreement.

The relationship between the metabolic rates and body mass (in this study *R* and *U*) is a classic physiological issue and has been discussed extensively in the past. Kleiber (1932) supported that an animal's metabolic rate is proportional to its body

mass raised to the power of 3/4, and this relationship has been found to hold true for the living organisms with size differences of more than 20 orders of magnitude. Bertalanffy (1957) distinguished three metabolic types according to the relationship between metabolic rate and body size. In the first type, the metabolic rate is proportional to a surface or the 2/3 power of the body mass (b=0.67); in the second type, the metabolic rate is proportional to the body mass (b=1); and, in the third type, the metabolic rate is intermediate between proportionality to body mass and proportionality to surface area ($0.67<b<1$). Recently, West et al. (1997) developed a general model that describes how essential materials are transported through space-filling fractal networks of branching tubes and derived Kleiber's 3/4-power scaling law. However, the theory of West et al. (1997) has been criticized by many researchers, who question the initial assumptions (Whitfield 2001). Dodds et al. (2001) re-analyzed published data sets dating back to Kleiber's original and concluded that the scaling exponents in most of them were statistically indistinguishable from 2/3. Darveau et al. (2002) presented a multiple-causes model of allometry, where the exponent b is the sum of the influences of multiple contributors to metabolism and control, which also supported the 3/4-power law for basal metabolic rate (but not for maximal metabolic rate). In *O. vulgaris*, we found b_R=0.901 and b_U=0.896, and the corresponding 95% confidence intervals were $0.862< b_R <0.939$ and $0.824< b_U <0.968$. Thus, it was established that *O. vulgaris* does not follow either the 3/4 law or the 2/3 law, and, speaking in terms of Bertalanffy's classification, it belongs to the third type. The b_R and b_U values did not vary with temperature.

For *Octopus cyanea*, Maginniss and Wells (1969) found b_R=0.833±0.031. *O. cyanea* is very similar to *O.vulgaris* in appearance, in its general behavior and habits and in its glandular physiology (Maginniss and Wells 1969; Wells and Wells 1969; Yarnall 1969; Wells and Wells 1970). However, when processing the raw weight and oxygen consumption values presented by Maginniss and Wells (1969) and comparing the results of the two studies, we found that the two b_R coefficients for *O. vulgaris* and *O. cyanea* differ significantly (df=132, t=2.608, p<0.02). Both species, though, have b_R coefficients that are significantly higher than 3/4 (Kleiber's law). Segawa and Hanlon (1988) found for young *O. maya* the same scaling exponent (b_R=0.900) as our value for *O. vulgaris*. Among other cephalopods, the b_R factor has been reported to be 0.87 for *Illex illecebrosus* (Kao 1970), 0.954 for *I. illecebrosus* (DeMont and O'Dor 1984), 0.771-0.987 for *Sepia officinalis* (Johansen et al. 1982), 0.910 for *Lolliguncula brevis* (Segawa and Hanlon 1988), 0.848 for *Loligo forbesi* (Segawa and Hanlon 1988) and 0.929 for *Sepioteuthis lessoniana* (Segawa 1995). Thus, it seems that cephalopods generally have b_R coefficients higher than 3/4 (Kleiber's Law).

Wells et al. (1983a) found b_R=0.72 (R^2=35%) for *O. vulgaris*, during recordings in 48 octopuses with body masses of 382±136g (mean ± standard deviation). We consider the value in the present study to be more precise, as the body mass range (382±136g) reported by Wells et al. (1983a) is more narrowly distributed than that reported in the present study (520.8±495.6); wide variations in b values are often due to a limited size range (Carvalho and Phan 1997).

The O/N atomic ratio is extensively used as a metabolic index. During starvation, the O/N ratio is clearly linked to the availability of energy reserves and the use of body protein (Mayzaud and Conover 1988). Using theoretical computations, it can be shown that pure 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). Under natural feeding conditions,

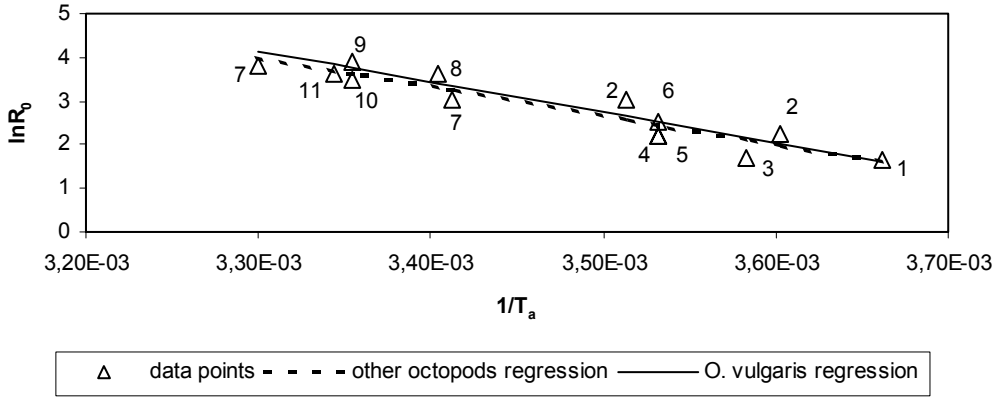

Fig. 4: A plot of log-transformed oxygen consumption rates (log*R*), normalized to a body mass of 350g (with scaling factor 0.901), as a function of $1/T_a$ (T_a is the absolute temperature in degrees Kelvin) for several octopod species. The least squares line that fits the data and the regression found in this study for *Octopus vulgaris* (Eq. 9) normalized to *M*=350g are also given. 1. *Paraledone charcotti* (Daly and Peck 2000), 2. *Eledone cirrhosa* (Daly and Peck 2000), 3. *Octopus californicus* (Seibel and Childress 2000), 4. *Octopus bimaculoides* (Seibel and Childress 2000), 5. *Octopus micropyrsus* (Seibel and Childress 2000), 6. *Octopus dofleini* (O'Dor and Wells 1987), 7. *Octopus briareus* (Borer and Lane 1971), 8. *Octopus vulgaris* (Wells et al. 1983a), 9. *Octopus cyanea* (Van Heukelem 1976), 10.*Octopus maya* (Segawa and Hanlon 1988), 11. *Octopus cyanea* (Maginniss and Wells 1969).

changes in the O/N ratio will not only reflect the type of metabolic substrate oxidized, but also the metabolic relationships between the animals and their food supply. The actual O/N ratio seems to depend not so much on the chemical composition of the food, as on the use made by the animals of each biochemical fraction assimilated. The low O/N ratio found in our study (=5.5) is indicative of protein-dominated catabolism. However, this value depends on the type of food, and different O/N ratios might arise under different feeding conditions. The values reported by Boucher-Rodoni and Mangold (1985) for two individuals fed with crabs are higher (O/N = 7.19 and 14.15), mainly because of their elevated *R* values.

We found the O/N atomic ratio to be independent of body mass, so octopuses with mass values between 20g and 1000g do not seem to have obvious differences in their metabolic relationships dependent upon food supply. We also found no significant dependence of the O/N ratio on temperature in the range of 15.5-26^{o}C, indicating no serious differences in the type of metabolic substrate oxidized or the metabolic pathways within this temperature range.

Wells et al. (1983a) have estimated a Q_{10} of 1.77 for *O. vulgaris* at a temperature range of 18-24^{o}C, which is slightly lower than our value for that range (Fig. 3). Borer and Lane (1971) found a Q_{10} of 2.18 for *O. briareus* at temperatures between 20-30^{o}C which is in agreement with our values for *O. vulgaris* (Fig. 3).

The dependence of the metabolic rate of *O. vulgaris* on temperature is contained in the term e^{-E_i/kT_a} of Eq.2. The E_i values should fall within the range of measured activation energies for metabolic reactions, which is between 0.2 and 1.2 eV, with an average of approximately 0.6 eV (Gillooly et al. 2001). Indeed, our estimations for E_i fell within this range, and $E_{i(R)}$ was actually found equal to the average value of 0.6 eV. Gillooly et al. (2001) evaluated metabolic rate data as a function of temperature for a variety of organisms (aerobic microbes, plants,

multicellular invertebrates, fishes, amphibians, reptiles, birds, and mammals), and they estimated average activation energies ranging between 0.41 and 0.74 eV, suggesting that as a first approximation the mass-scaled metabolic rates of all organisms are a single, general function of temperature. The results of this study are in agreement with that statement. Furthermore, to compare our estimation of $E_{i(R)}$ with data for other octopuses, we plotted mass-normalized oxygen consumption rates [$\ln R_0$ (with b_R=0.901)] as a function of $1/T_a$ using data from the literature (Fig. 4). From the slope of the line fitted with least squares to the data, an activation energy of E_i=0.56±0.13 (95% confidence interval) was calculated (had we used b_R=0.75 for the mass-normalization of the data, we would have found E_i=0.58±0.15, which is statistically equal to the result with b_R=0.901). This activation energy does not differ significantly from the corresponding value found for *O. vulgaris*, indicating that the metabolic rates of octopod species in a very similar way depend on temperature. Thus, the temperature dependence of the metabolic rate of *O. vulgaris*, as estimated in this study by Eq. 5, may be generalized (as a first approximation) for other octopod species.

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