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Is there a universal allometric scaling of metabolism?

Cephalopods as a case study.

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Resumo

Entre as inúmeras questões relativas à fisiologia dos animais uma das mais prementes prende-se com a forma como o metabolismo de um organismo varia de acordo com o seu peso. A dependência do metabolismo (Y) face à massa do organismo (M) segue a equação alométrica $Y = b_0M^b$ onde b_0 é a constante de normalização, específica para cada *taxon* e b é o coeficiente de escalonamento. A Teoria Ecológica do Metabolismo (TEM) postula que a temperatura e a riqueza de espécies estão correlacionadas, uma vez que, a temperatura aumenta as taxas de mutação (devido à aceleração de processos bioquímicos), acelerando por sua vez a evolução molecular e levando a um aumento da taxa de especiação em zonas mais quentes. No entanto, actualmente, diversas premissas desta teoria têm sido contestadas. Uma das premissas desta teoria é o facto da existência de escalonamento metabólico universal (lei dos 3/4) e os mecanismos que estão subjacentes a este, o qual reúne pouco consenso científico. Segundo esta teoria todos os organismos têm em comum as mesmas relações de escalonamento alométricas e que o modelo geral da origem das taxas metabólicas alométricas descreve como os materiais essenciais às células são transportados através da vasta rede ramificada de distribuição de energia, como são os vasos capilares, e que é esta rede a responsável pela atribuição do coeficiente de escalonamento de três quartos, ou o equivalente logaritmizado para massa e taxa metabólica, -0.25. Modelos teóricos baseados nas restrições geométricas da rede de abastecimento celular são previsivelmente limitados para animais aquáticos de corpo mole, tais como os cefalópodes, uma vez que podem efetuar trocas gasosas e de matéria orgânica através da superfície corporal.

Em resposta à premissa universal da lei dos três quartos, foi proposto que, após a correção para a massa corporal e temperatura, todos os organismos de um dado tamanho partilham a mesma taxa metabólica. Isto implica que a evolução e a ecologia possuem um papel mínimo na variação metabólica. Esta generalização ecológica não é suportada nem pela heterogeneidade nos expoentes de escalonamento nem pelas constantes de normalização encontradas na literatura. Estas sim, parecem expressar a variação de mecanismos biológicos envolvendo considerações evolucionárias e ecológicas.

A Classe Cephalopoda encontra-se representada na lista de estudos de escalonamento metabólico que integram uma elevada heterogeneidade das constantes de normalização e coeficientes de escalonamento. Estes invertebrados possuem características que os tornam comparáveis a vertebrados, como sistema nervoso altamente desenvolvido,

sistema circulatório fechado e um grande repertório de comportamentos. A fisiologia de cefalópodes bentónicos e nectobentónicos está amplamente estudada. É um habitat que promove estilos de vida menos exigentes a nível energético, uma vez que, oferece melhores oportunidades de esconderijo e camuflagem. No que diz respeito à fauna teutológica do Oceano Atlântico Este, as espécies mais comuns são o polvo comum *Octopus vulgaris* e o choco comum *Sepia officinalis.*

No presente trabalho foi efectuado um estudo ontogenético da variação das taxas metabólicas, de ambas as espécies acima referidas, a três temperaturas diferentes, com o intuito de aferir se os coeficientes de escalonamento e o modo como o factor extrínseco – temperatura - pode afectar o escalonamento metabólico.

Tal como esperado, registou-se o aumento das taxas metabólicas com o aumento da temperatura e alterações metabólicas ontogenéticas significativas, i.e. maiores necessidades energéticas dos organismos recém-eclodidos estudados. À nascença, os polvos são paralarvas pelágicas, habitando a zona epipelágica do oceano, onde é necessária a utilização do modo de locomoção caraterístico dos cefalópodes - propulsão a jacto. Este modo de locomoção é energeticamente pouco eficiente quando comparado com o modo de locomoção ondulatório/oscilatório dos peixes, requerendo taxas metabólicas aproximadamente dez vezes superiores às taxas metabólicas de polvos adultos. Aquando do assentamento das paralarvas a taxa metabólica destas decresce, uma vez que no ambiente bentónico a necessidade de utilização da propulsão a jacto é drasticamente reduzida, e os polvos juvenis e adultos utilizam maioritariamente modos de locomoção menos dispendiosos. Comparativamente, os chocos, desde a sua eclosão até à fase adulta, não sofrem grandes alterações na sua morfologia e fisiologia, assemelhando-se a "pequenos" adultos aquando da eclosão, e adoptando imediatamente os comportamentos típicos daqueles. Como tal, a exigência metabólica dos chocos recém eclodidos é muito inferior à dos polvos recém eclodidos, sendo, no entanto, superior à dos chocos juvenis. Isto deve-se ao facto dos cefalópodes serem organismos com taxas de crescimento muito elevadas no início da sua vida, sendo estas mais elevadas do que no restante ciclo de vida.

As diferenças entre as taxas metabólicas de juvenis de choco e polvo revela a maior necessidade de gasto de energia por parte dos chocos devido ao seu estilo de vida nectobentónico, que obriga à manutenção da flutuabilidade através da siba, e do constante movimento das aletas.

O coeficiente de escalonamento de ambas as espécies revela uma alometria negativa (b < 0) que reflecte os seus estilos de vida, menos activos que aquele das espécies pelágicas de cefalópodes. No presente estudo os coeficientes de escalonamento enquadram-se na gama de valores abrangida pela TEM, apoiando assim a universalidade desta Teoria.

No presente estudo não foi, no entanto, incluída a análise das taxas metabólicas e coeficientes de escalonamento da lula europeia *Loligo vulgaris* devido à impossibilide da amostragem de especimens.

Outros estudos sobre o escalonamento metabólico de cefalópodes permitem verificar a existência de diferentes relações de escalonamento entre lulas musculares ativas e os cefalópodes do presente estudo. Naquelas, o metabolismo específico (por grama de indivíduo) é quase independente do seu tamanho, e por isso, parece estar relacionado com o dispêndio massivo de energia na locomoção a jacto.

Os proponentes da TEM têm vindo a focar demasiada atenção na influência da massa de um organismo e temperatura nas taxas metabólicas, deixando de parte a ecologia e evolução, factores importantes que moldam o ciclo de vida de qualquer espécie. A quantidade de provas disponíveis respeitando o metabolismo de cefalópodes permitem-nos duvidar da existência de tal lei. No entanto, as evidências referidas abordam sobretudo lulas com hábitos pelágicos. Pelo contrário, os resultados aqui discutidos, tendo em conta o ciclo de vida de duas espécies de cefalópodes que habitam o bentos mostraram expoentes de escalonamento que representam as necessidades energéticas deste habitat, apoiando a universalidade da TEM.

Palavras-chave: Teoria Ecológica do Metabolismo, escalonamento metabólico, cefalópodes, alometria

Is there a universal allometric scaling of metabolism? Cephalopods as a case study.

Abstract

The aim of the present study was to follow the ontogenetic metabolic scaling, of two coastal cephalopods with different life strategies, the benthic common octopus *Octopus vulgaris* and the nektobenthic common cuttlefish *Sepia officinalis*. Oxygen consumption rates were measured for hatchlings, juveniles and adult stages exposed to three different temperatures. Our results show significant differences between the metabolic rates of *O.vulgaris* hatchlings and adults, reflecting the morphological and locomotory changes that this species undergoes throughout ontogeny. Significant inter-specific differences in the metabolic rates were also obseved, with the nektobenthic cuttlefish exhibiting higher metabolic, possibly associated with a more energy demanding life strategy. But most importantly, our findings have shown little deviation from the main premise of the Metabolic Theory of Ecology, the 3/4-power law, since both species' scaling exponents fell closer to -0.25.

Keywords: Metabolic Theory of Ecology, metabolic scaling, cephalopods, allometry

Introduction

Since the early years of modern biology scientists have tried to understand how an organism's metabolism is affected by its body mass. Metabolism is the process by which energy is exchanged between the organism and its environment (Gillooly *et al.* 2001), also comprising the transformation and allocation of such energy that occurs within the organism (Brown *et al.* 2004). The relationship between metabolic rate (Y) and body mass (M) is known to follow the allometric equation $Y = b_0 M^b$ (Rosa *et al.* 2009). In this equation b_0 is the normalization constant, which expresses the energetic costs of an organism (Demetrius 2006) to be fitted empirically (Brown *et al.* 2004), and b defined as the scaling exponent, that is, the ratio of variation in metabolic rate accompanying changes in body mass (Demetrius 2006). The value of the scaling

exponent, often falls near one-quarter power (b= -0.25; Rosa *et al.* 2009), being widely accepted as 3/4 (Hemmingsen 1960; Kleiber 1961; Brody 1945; McMahon and Bonner 1983; Peters 1983; Schmidt-Nielsen 1984; Blaxter 1989; Brown *et al.* 2004; Savage *et al.* 2004). The quarter power metabolic scaling (body mass increasing fourfold, while log metabolic rate increases only threefold; Glazier 2006) is, to some, a universal biological law (West *et al.* 1997; West and Brown 2004), since in literature there is a visible pattern regarding this scaling exponent across habitats and *taxa* (Hemmingsen 1960; Gillooly *et al.* 2001). The belief that this 3/4-power law is universal was supported by resource and transportation models that claim to predict universal quarter-power scaling for metabolic rates and other biological processes (West *et al.* 1997; Banavar *et al.* 1999; Brown *et al.* 2004; Savage *et al.* 2004; West and Brown 2004). Nevertheless it has been shown that both, the normalization constant (White *et al.* 2006; Seibel 2007) and the scaling exponent varies widely among different physiological states, both within and between species (Bokma 2004; Glazier 2005; 2008; 2009 Niver and Scharlemann 2005; Weibel 2005; White and Seymour 2005; White *et al.* 2006; Seibel 2007; Makarieva *et al.* 2008, Rosa *et al*. 2009).

From this commonality of metabolic scaling arose the Metabolic Theory of Ecology (MTE), based upon the principle that an organism's growth, survival and reproduction is determined by its metabolic rate and limited by the organism's capacity of resource uptake due to constraints of the fractal-like branching networks of fuel delivery (Brown *et al.* 2004).

The validity of this proposed single cause (constraints of resource uptake) is not consensual among physiologists, since throughout time, such networks have evolved in order to meet the cells' energy requirements, even in maximal effort (Bishop 1999; Weibel 2002). Thus, oxygen supply (used as proxy of metabolic rate) does not seem to adequately explain the size dependence of an organism's metabolic rate (Seibel 2007).

Gillooly *et al.* (2001), following the universality of the quarter-power law postulated by West *et al.* (1997) proposed that after due correction for body size and temperature, all organisms with the same body mass share the same metabolic rate. Yet, the commonality of scaling exponents in literature may be misleading, because the compilation of allometric relations made by Peters (1983) was mainly (78%) about vertebrates, even though most animals are invertebrates (Glazier, 2005). Such fact does not support the proposal of Gillooly *et al.* (2001), since it implies that evolution and

ecology play minor roles in determining an organism's metabolic rate (Rosa *et al.* 2009).

Amidst the heterogeneity of normalization constants and scaling exponents problematic reported by Seibel (2007), lies the cephalopod class. Cephalopods are a very diverse group of marine invertebrates inhabiting the ocean from the poles to the tropics, from shallow depths to the abyssal trenches, exhibiting varied life strategies, that result in a diversity of metabolic rates, unusual in the animal kingdom (Rosa *et al.* 2008 a, b; 2009; Rosa and Seibel 2008, 2010). Cephalopods exhibit features that make them comparable to vertebrates, such as a complex behavior repertoire, a highly developed nervous system, a closed blood convection system, with low blood volume and a high oxygen extraction efficiency resulting from their ability to slow down the ventilatory system and maintaining a high oxygen exchange surface and high circulatory rate (Wells and Wells 1983, 1985, 1986; Wells 1988; Eno 1994), and also the high concentration of hemocyanin in their blood, which is pumped by two branchial and one powerful systemic heart (Schipp 1987; Wells and Smith, 1987; Wells 1992; Pörtner and Zielinski 1998). The physiology of some benthic and nektobenthic cephalopods has been well studied (Wells and Wells 1982; O'Dor and Wells 1987; Wells 1988; Wells and Wells 1991; Seibel and Childress 2000; Melzner *et al.* 2006). The benthic habitat does not require high locomotory efficiency, since they have more crypsis and refuge opportunities (Seibel and Childress 2000). The benthic *Octopus vulgaris* (Lamarck, 1798) is the most studied species of the *Octopus* genus. It inhabits mostly shallow coastal habitats of the temperate, subtropical and tropical Atlantic, Indian and Pacific Oceans, occurring also in the Mediterranean Sea (Mangold 1998). The nektobenthic *Sepia officinalis* Linnaeus 1758 is found on the continental shelf of the Eastern Atlantic Ocean and also Mediterranean Sea (Boletzky, 1983; Guerra and Castro 1988) being the most common species of the *Sepia* genus occurring in these waters.

No ontogenetic studies on metabolic scaling have ever been performed on *O.vulgaris*, and *S. officinalis*. Hence, the aim of this study is to investigate, for the first time, the ontogenetic metabolic scaling of coastal cephalopods with two distinct life strategies, namely the benthic common octopus *O. vulgaris* and the nektobenthic common cuttlefish *S. officinalis* in order to test the universality of the 3/4-power law and to investigate how extrinsic effects, such as temperature may affect metabolic scaling.

Materials and Methods

Specimen collection and stocking conditions

Egg clutches of *O. vulgaris* and *S. officinalis* eggs were collected near Cascais, in April 2012 (cuttlefish) and July (octopus) and brought to Guia's Marine Laboratory. Eggs were placed in aquaria at 20ºC until hatching.

Juvenile and adult octopuses ranging from 37 to 283 g total weight were acquired from local fishermen between December 2011 and October 2012. Juvenile cuttlefish ranging from 3.8 and 26 g total weight were captured in Sado estuary during October 2011 and July 2012. During transportation, organisms were kept in 60 L containers filled with seawater aerated through the use of portable air pumps. Upon arrival at the laboratory, they were placed and acclimated into 540 L rectangular tanks, within a recirculating aquaculture system (RAS), equipped with mechanic, biologic and physical filtration as well as UV disinfection until they were used for metabolic rates quantification. A total of 22 octopus specimens across, three orders of magnitude of mass (wet weight; 0.001-0.01 g, 10-100 g, 100-1000 g), and 33 cuttlefish specimens, across three orders of magnitude of mass (wet weight; 0.1-1.0 g, 1-10 g, 10-100 g) were used.

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Determination of metabolic rates

Metabolic rates (oxygen consumption rates) were performed at different temperatures for *O. vulgaris* (10 ºC, 15 ºC and 20 ºC) and *S. officinalis* (15 ºC, 20 ºC and 25 ºC).

The experimental temperatures used to quantify the metabolic rates of hatchlings were the same used for juveniles and adults. These temperatures were chosen to reflect the normal thermal amplitude that adults face throughout the year. For instance, and contrary to octopods, the common cuttlefish is found more offshore during winter and, in early spring, it makes extensive horizontal migrations to spawn in neritic zones, including the Sado Estuary, where it faces temperatures above 25ºC (Baptista *et al.* 2012).

The metabolic rates of cuttlefish and octopus hatchlings were measured according to the methods described in Seibel *et al.* (2007) and Rosa *et al.* (2009).

Hatchlings were placed in gas-tight glass syringes with 3 ml (for octopus) and 10 ml (for cuttlefish) of filtered (0.2 μm) and UV sterilized seawater (35 psu). Seawater used in the syringes was aerated until saturation, in order to prevent eventual oxygen level

decrease, as suggested by Ikeda *et al*. (2002). Simultaneously and for each experiment, a control syringe (containing only the previously mentioned water) was used to detect, and if necessary, correct any possible bacterial respiration.

A total of 8 syringes were used at each experimental temperature, and these were placed in a temperature controlled water bath (Lauda, Lauda-Königshofen, Germany), with the duration of respiratory runs varying between 90 and 360 minutes for octopus and 40 and 60 minutes for cuttlefish.

At the end of each run, oxygen levels were measured through the extraction of a water sample from the incubation syringes using a Hamilton gas-tight 500 ml syringe, and subsequent injection into a micro-respiratory chamber Mc 100 Microcell (Strathkelvin, North Lanarkshire, Scotland) where oxygen concentrations were recorded using a Clarke-type O_2 electrode connected to a multi-channel oxygen interface (Model 928, Strathkelvin, North Lanarkshire, Scotland). The oxygen electrode and the micro-respiratory chamber were always kept at the experimental temperatures (Marsh and Manahan 1999; Seibel *et al.* 2007).

Regarding the juvenile and adult stages, the metabolic rates were quantified in a flow-thru respirometry system consisting of a 73 L column connected to respirometer chambers with different volumes (from 0.25 to 6.8 L capacity), selected to accommodate differently sized animals. Specimens were allowed to acclimate during an initial period of 2 h before starting the actual measurements of their oxygen consumption (Rosa and Seibel 2008c; 2010). The seawater used in the experimental procedures was mechanically filtered (0.2 μm), UV sterilized and continuously aerated to maintain oxygen saturation. Depending on the size of the chamber, a peristaltic pump (Masterflex L/S, Model no 7524-45, Cole-Parmer Instrumental Company, IL, USA) or a water pump (used for larger chambers) was used to circulate water within the flow-thru (column-chamber-column) system.

Oxygen concentrations were recorded at the exit of each chamber with a Clarke-type O_2 electrode connected to a 929 Oxygen Interface (Strathkelvin Instruments). Additionally one electrode directly connected to the column (i.e. not connected to a chamber) was used as a control for oxygen concentration. The system was calibrated using oxygen-saturated seawater before each run and at each experimental temperature (using the correspondent maximum dissolved oxygen concentration value; Rosa and Seibel 2010). The experimental temperatures used to quantify the metabolic rates of the juvenile and adults were the same used for hatchlings.

After the animal's stress phase, the duration of the runs (and data recording) was variable, but a minimum duration of 6 hours was established for every specimen tested. After the experimental runs, all specimens were weighed.

Statistical analysis

Oxygen consumption rates were evaluated in relation to wet weight using simple linear regressions. Regression slopes were considered to be significant when their slopes differed from 0 at the 95% confidence level. ANCOVA was also used to compare energy expenditure rates of *O. vulgaris* and *S. officinalis* throughout their ontogeny and at the different temperatures used. All statistical analyses were performed with the software STATISTICA^{TM} 6.1. (Statsoft, Inc., Tulsa, OK 74104, USA).

Results

The regression analysis showed that the mass-specific metabolic rates of *O. vulgaris* were negatively affected by body mass in all three temperatures used (Fig. 1, Table 1). When controlling the effect of size on mass-specific metabolic rates, temperature was shown to be a significant factor, affecting the routine metabolic rates of octopus specimens (ANCOVA: $F = 87.24$; $p = 0.000$).

The smallest *O. vulgaris* (i.e. a hatchling) weighed 1.89×10^{-3} g and had an oxygen consumption rate of 11.989 μmol O_2 g^{-1} h^{-1} at 10 ºC, 16.956 μmol O_2 g^{-1} h^{-1} at 15 ºC and 23.979 μmol O_2 g^{-1} h^{-1} at 20 ºC. The largest specimen of *O. vulgaris* weighed 238.0 g and had an oxygen consumption rate of 0.978 μmol O_2 g^{-1} h^{-1} at 10 ºC, 1.866 μmol O_2 g^{-1} h^{-1} at 15 ºC and 3.295 μmol O_2 g^{-1} h^{-1} at 20 ºC. However, temperature did not affect the scaling relationship between mass-specific metabolic rate and body mass (ANCOVA: $F = 2.46$; $p = 0.098$).

Regression analysis performed on *S. officinalis* showed that their mass-specific metabolic rates were affected by body mass in all three temperatures used (Fig. 2, Table 1).
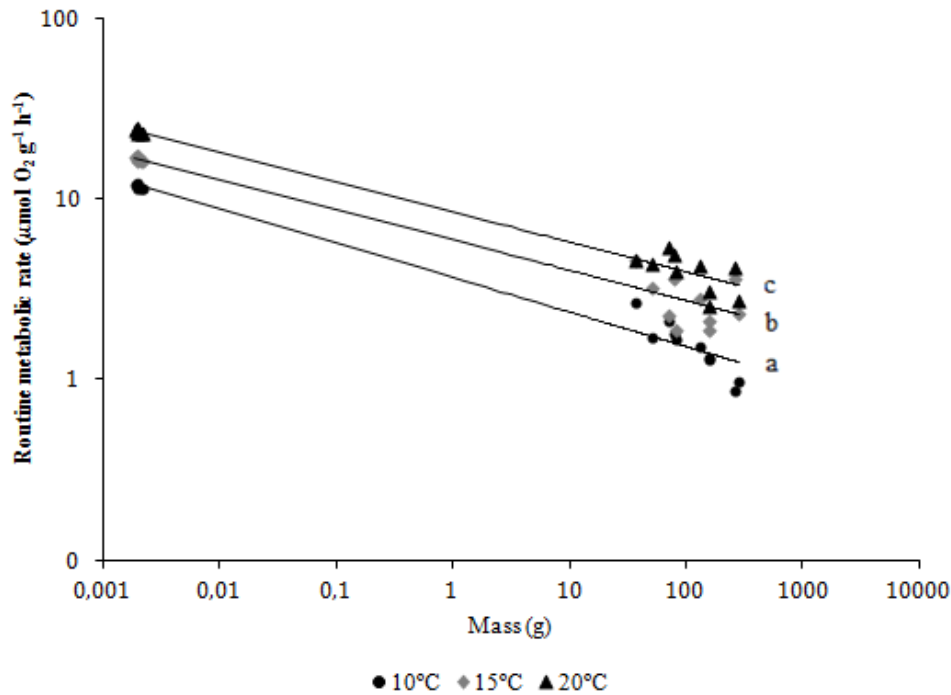
Figure 1. Mass-specific oxygen consumption rates (μmol O_2 g^{-1}h^{-1}) as a function of body size of *O. vulgaris* at the three experimental temperatures. Different letters represent significant differences between temperatures ($p < 0.05$). The equations for the regressions are presented in Table 1.

Regarding *S. officinalis*, and at 15 °C, the smallest hatchling tested weighed 0.182 g and had an oxygen consumption rate of 8.623 μmol O_2 g^{-1} h^{-1} and the larger specimen weighed 26.0 g and had an oxygen consumption rate of 6.360 μmol O_2 g^{-1} h^{-1}. At 20 °C, the smallest specimen weighed 0.156 g and had an oxygen consumption rate of 16.676 μmol O_2 g^{-1} h^{-1} and the largest weighed 18.0 g and had an oxygen consumption rate of 5.209 μmol O_2 g^{-1} h^{-1}. At 25 °C, the smallest weighed 0.098 and had an oxygen consumption rate of 16.062 μmol O_2 g^{-1} h^{-1} and the largest weighed 13.0 g and had an oxygen consumption rate of 8.299 μmol O_2 g^{-1} h^{-1} at 20 °C. When controlling the effect of size on mass-specific metabolic rates, temperature was shown to be a significant factor, affecting the routine metabolic rates of cuttlefish specimens (ANCOVA: $F = 39.93$; $p = 0.000$). However temperature did not affect the scaling relationship between mass-specific metabolic rate and body mass (ANCOVA: $F = 2.09$; $p = 0.142$).
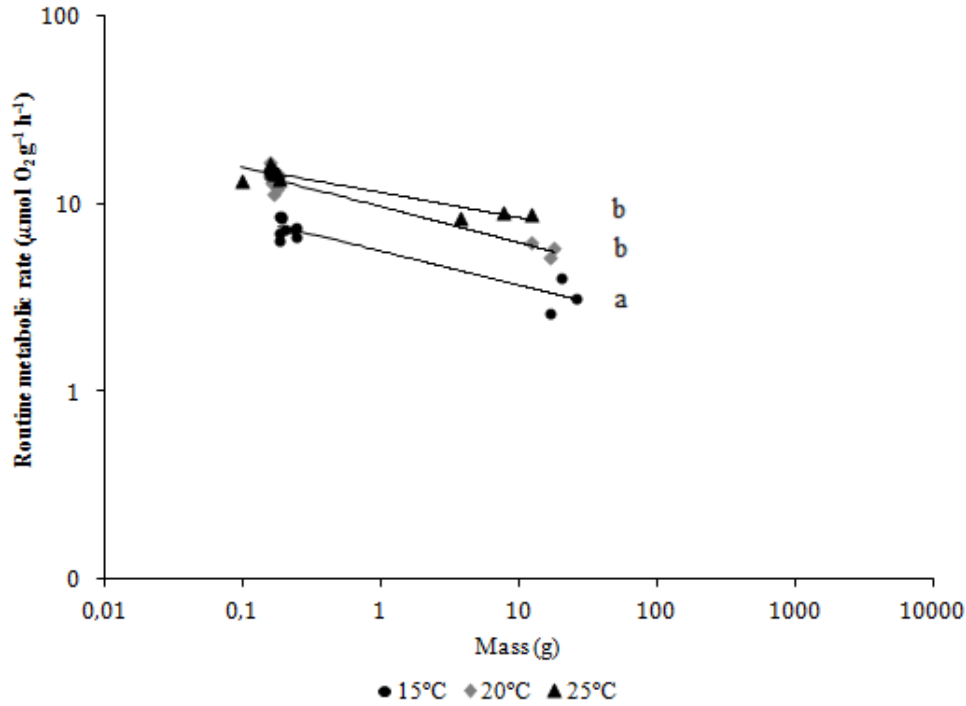
Figure 2. Mass-specific oxygen consumption rates (μmol O_2 g^{-1} h^{-1}) as a function of body size of *S. officinalis* at the three experimental temperatures. Different letters represent significant differences between temperatures ($p < 0.05$). The equations for the regressions are presented in Table 1.

Table 1. Size range, mass-specific oxygen consumption rates (μmol O_2 g^{-1} h^{-1}) as a function of body size and regression parameters for *O. vulgaris* and *S. officinalis* at the different experimental temperatures.

Oxygen consumption	*O. vulgaris*			*S. officinalis*		
($MO_2 = aM^b$)	10 ºC	15 ºC	20 ºC	15 ºC	20 ºC	25 ºC
Mass (g)	0.002 - 283	0.002 - 283	0.002 - 283	0.182 - 26	0.156 - 18	0.098 - 13
Rate (μmol O_2 g^{-1} h^{-1})	0.978 - 11.989	1.866 - 16.956	3.295 - 23.979	6.360 - 8.623	5.209 - 16.676	8.299 - 16.062
a	3.6567	5.8659	8.4412	5.573	9.6231	11.429
b	-0.192	-0.168	-0.166	-0.182	-0.192	-0.134
n	16	17	17	12	10	13
r^2	0.974	0.946	0.972	0.901	0.934	0.884
p-value	0.000	0.000	0.000	0.007	0.004	0.000

When controlling the effect of size on the mass-specific metabolic rates of each species (Fig. 3), it was shown that species identity affects mass-specific metabolic rates

(ANCOVA: $F = 8492.72$; $p = 0.000$), and that species identity also affects the relationship between mass-specific metabolic rates and body mass (ANCOVA: $F = 14.63$; $p = 0.001$).
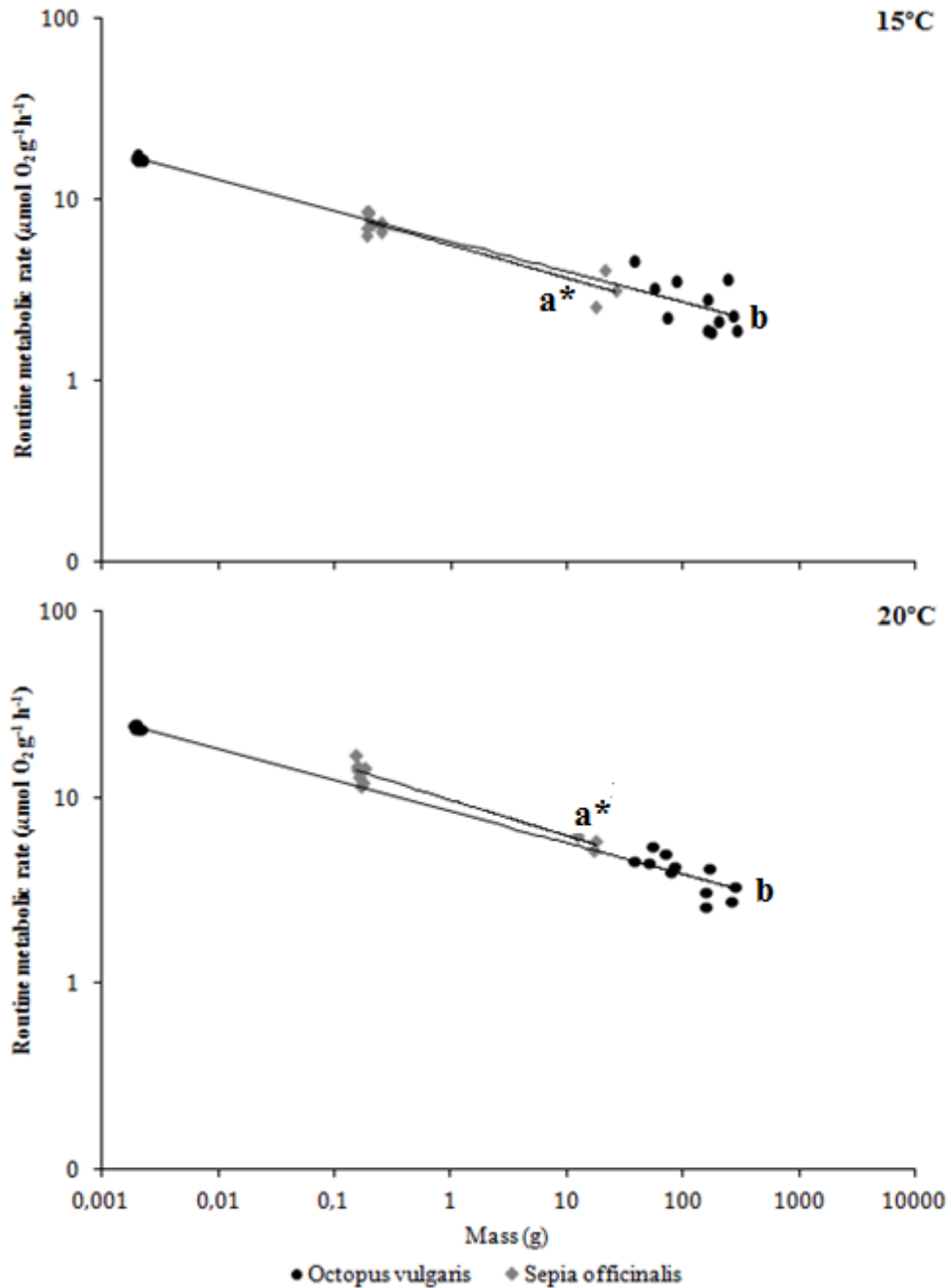

Figure 3. Comparison of the mass-specific oxygen consumption rates (μmol O_2 g^{-1} h^{-1}) as a function of body size between *O. vulgaris* and *S. officinalis* at 15 °C and 20 °C. Different letters represent significant differences between metabolic rates of both species by controlling the effect of size. Asterisks represent significant differences regarding scaling exponents of both species.

Discussion

The universality of the Metabolic Theory of Ecology (MTE) has long been debated. As otherwise noted by Rosa *et al.* (2009) several animals, both terrestrial (Darveau et al. 2002; Brown *et al.* 2004; Glazier 2005) and aquatic (Gillooly *et al.* 2001; Clarke 2006; Glazier 2005) exhibit metabolic scaling to the power of -0.25. Concordantly, the scaling exponents derived from both species of the present study fall within the same range (i.e. *O. vulgaris*: 10 ℃, b = -0.192; 15 ℃, b = -0.168; 20 ℃, b = -0.166; S. *officinalis*: 15 ℃, b = -0.182; 20 ℃, b = -0.192; 25 ℃, b = -0.134). Seibel and Drazen (2007) found that the variation in normalization constants among the benthic species within a phylum is not as pronounced as the variation of the normalization constants among the pelagic species of the same phyla reflecting the more limited range of activity levels on the benthos (Seibel and Childress 2000).

Large differences in energy expenditure rates were found between the hatchlings and adults of *O. vulgaris*, with hatchlings displaying over ten times higher oxygen consumption rates than adults, reflecting higher metabolic rate requirements during the critical pelagic paralarvae phase of their lives. *O. vulgaris* paralarvae rely on jet propulsion as the means of locomotion (Bartol 2009; Pimentel *et al.* 2012) and most likely to find prey in the epipelagic zone. Jet propulsion is widely known to be energetically inefficient representing a large toll on the species' energy demand when compared to the undulatory/oscillatory swimming of fishes (O'Dor and Webber 1986; Rosa and Seibel 2008c; Rosa *et al.* 2009). Moreover, in cephalopods, a pelagic lifestyle has been shown to be associated with a high energetic demand and isometric scaling, as observed for gonatid squids (Rosa *et al.* 2009). Squids are known to be active, highly energy demanding predators of the pelagic realm, having high mass independent metabolic rates and having isometric metabolic scaling (b ~ 0; Seibel 2007; Rosa *et al.* 2009), resulting in an energetically expensive lifestyle throughout ontogeny (Rosa *et al.* 2009). The pelagic lifestyle is a very demanding and energetically expensive mode of life, when compared to the calmer, with higher refuge opportunities (Seibel and Childress 2000), benthic and nektobenthic lifestyle.

When octopus hatchlings leave the water column and settle, there is no need for regular use of jet propulsion (i.e. to maintain buoyancy), which together with the existence of more crypsis and refuge opportunities in the benthic environment results in a decrease of the metabolic rate (Seibel and Childress 2000).

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Glazier (2005) resurrected a tentative classification of intraspecific scaling based on Bertalanffy's (1957) notion of different metabolic types. Type III is listed as nonlinear, comprising an ontogenetic shift from near isometry in early life to negative allometry in adulthood. *O. vulgaris*, with its distinct life phases would expectedly fit this type. In our study, however, we could not find isometry in the early life stage. Even if octopus hatchlings don't undergo major changes, they shift between two very different habitats (i.e. migrate from the epipelagic to the benthic realm), a transition naturally associated with biochemical changes hence making them suitable for nonlinear metabolism.

On the other hand, differences in energy expenditure between hatchlings and juveniles of *S. officinalis* were less pronounced given that, as hatchlings, *S. officinalis* are less energetically demanding. Cuttlefish do not show drastic developmental shifts throughout ontogeny, spending some periods of time in the water column but also dwelling on the bottom (nektobenthic life strategy) with large periods of resting (Aitken *et al.* 2005). Also, cuttlefish possess a cuttlebone, that facilitates their buoyancy (Denton and Gilpin-Brown 1961a, b; Webber *et al.* 2000).

The extrinsic effect here studied (i.e. temperature) was found to have a significant effect on the metabolic rate of the species studied, with increases in temperature resulting in increases in the mass-specific metabolic rates of both *O. vulgaris* and *S. officinalis*. This happens because temperature is one the main factors dictating the metabolic rate of an organism, consequently affecting biochemical processes taking place within the body, since enzymes are very sensitive to temperature fluctuations, oxygen consumption, and even growth (Gillooly *et al.* 2001; Pörtner and Knust 2007; Seibel and Drazen 2007).

In this study we did not find any significant effect of temperature on the metabolic scaling (i.e. scaling exponent) of these two cephalopod species. Yet, it is worth noting that other studies were able to do so, namely in the fish *Etheostema olmstedi* (Kumai 2006).

We argue that proponents of the MTE have focused too much attention on the influence of the body size disregarding the role of ecology and evolution, important factors that shape the life history of any species. The amount of available evidence concerning cephalopod metabolism gives us room to doubt the universality of such a law (Seibel et al. 1997; Seibel and Childress 2000; Seibel 2007; Seibel and Drazen 2007; Rosa et al. 2009; Rosa and Seibel 2010b; Rosa and Seibel 2010c). However, such evidence comes mostly from reports on squids with pelagic lifestyles. On the other hand, the findings

reported herein (acknowledging the type of life strategy of two species studied) support the universality of the MTE.

References

Aitken JP, O'Dor RK, Jackson GD (2005) The secret life of the giant cuttlefish Sepia apama (Cephalopoda): behaviour and energetic in nature revealed through radio acoustic positioning and telemetry (RAPT). J Exp Mar Biol Ecol 320:77–91

Banavar JR, Maritan A, Rinaldo A (1999) Size and form in efficient transport networks. Nature 399:130-132

Baptista M, Lopes VM, Pimentel MS, Bandarra N, Narciso L, Rosa R (2012) Temporal fatty acid dynamics of the octocoral Veretillum cynomorium. Comp Biochem Physiol B 161(2):178-87.

Bartol IK, Krueger PS, Stewart WJ, Thompsonn JT (2009) Pulsed jet dynamics of squid hatchlings at intermediate Reynolds numbers. J Exp Biol 212:1506–1518

Bertalanffy LV (1957) Quantitative laws in metabolism and growth. Q Rev Biol 32:217–231

Bishop CM (1999) The maximum oxygen consumption and aerobic scope of birds and mammals: getting to the heart of the matter. Proc R Soc B 266:2275-2281

Blaxter K (1989) Energy Metabolism in Animals and Man. Cambridge University Press, Cambridge

Bokma F (2004) Evidence against a universal metabolic allometry. Funct Ecol 18:184-187

Boletzky S (1983) *Sepia officinalis*. In: Boyle, P.R. (Ed.), Cephalopod Life Cycles, vol. 1. Academic Press, London, pp. 31–52

Brody S (1945) Bioenergetics and Growth. Reinhold Publishing Corp, New York

Brown JH, Gillooly JF, Allen AR, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771-1789

Demetrius L (2006) The origin of allometric scaling laws in biology. J Theor Biol 243:455-467

Denton EJ, Gilpin-Brown JB (1961a) The buoyancy of the cuttlefish, Sepia officinalis (L.). J Mar Biol Assess UK 41:319–342

Denton EJ, Gilpin-Brown JB (1961b) The effect of light on the buoyancy of the cuttlefish. J Mar Biol Assess UK 41:343–350

Eno CN (1994) The morphometrics of cephalopod gills. J Mar Biol Assoc UK 74:687-706

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248-2251

Glazier DS (2005) Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. Biol Rev 80:611-662

Glazier DS (2008) Effects of metabolic level on the body-size scaling of metabolic rate in birds and mammals. Proc R Soc Lond B 275:1405–1410

Glazier DS (2009) Activity affects intraspecific body-size scaling of metabolic rate in ectothermic animals. J Comp Physiol B 179:821–828

Guerra A, Castro BG (1988) On the life cycle of *Sepia officinalis* (Cephalopoda: Sepioidea) in the ria de Vigo (NW Spain). Cah Biol Mar 29:395–405

Hemmingsen AM (1960) Energy metabolism as related to body size and respiratory surfaces and its evolution. Rep Steno Mem Hosp Copenhagen 9:1–110

Ikeda Y, Okazaki J, Sakurai Y, Sakamoto W (2002) Periodic variation in Sr/Ca ratios in statoliths of the Japanese common squid *Todarodes pacificus* Steenstrup, 1880

(Cephalopoda: Ommastrephidae) maintained under constant water temperature. J Exp Mar Biol Ecol 273:161-170

Kleiber M (1961) The Fire of Life. An introduction to animal energetics. Wiley, New York

Kumai Y (2006) The effect of water temperature on the distribution and metabolic rate of fish species in streams on Cape Cod. Project report from Woods Hole University, Massachussets.

Makarieva AM, Gorshkov VG, Li BL, Chown SL, Reich PB, Gavrilov VM (2008) Mean mass-specific metabolic rates are strikingly similar across life's major domains: evidence for life's metabolic optimum. Proc Nat Acad Sci USA 105:16994–16999

Mangold K (1998) The Octopodinae from the eastern Atlantic Ocean and the Mediterranean Sea. In: Voss NA, Vecchione M, Toll RB, Sweeney MJ (eds) Systematics and biogeography of cephalopods, vol 1. Smithson Contrib Zool 586:521–528

Marsh AG, Manahan DT (1999) A method for accurate measurements of respiration rates of marine invertebrate embryos and larvae. Mar Ecol Prog Ser 184:1–10

McMahon TA, Bonner JT (1983) On Size and Life. Scientific American Books, New York

Melzner F, Bock C, Pörtner HO (2005) Critical temperatures in the cephalopod *Sepia officinalis* investigated using in vivo ^{31}P NMR spectroscopy. J Exp Biol 209:891-906

Moreno A, dos Santos A, Piatkowski U, Santos AMP, Cabral H (2009) Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia. J Plankton Res 31:73–91

Niven JE, Scharlemann JP (2005) Do insect metabolic rates at rest and during flight scale with body mass? Biol Lett 1:346–349

O'Dor RK, Webber DM (1986) The constraints on cephalopods: why squid aren't fish. Can J Zool 64:1591–1605

Peters RH (1983) The ecological implications of body size. Cambridge University Press, New York

Pimentel MS, Trübenbach K, Faleiro F, Boavida-Portugal J, Repolho T, Rosa R (2012) Impact of ocean warming on the early ontogeny of cephalopods: a metabolic approach. Mar Biol 159:2051–2059

Pörtner HO, Hardewig I, Peck LS (1999) Mitochondrial function and critical temperature in the Antarctic bivalve, *Laternula elliptica*. Comp Biochem Physiol A 124:179-189

Rosa R., Dierssen HM, Gonzalez L, Seibel BA (2008a) Ecological biogeography of cephalopod mollusks in Atlantic Ocean: historical and contemporary causes of coastal diversity patterns. Global Ecol Biogeogr 17(5):600-610

Rosa R, Dierssen HM, Gonzalez L, Seibel BA (2008b) Large-scale diversity patterns of cephalopods in the Atlantic open ocean and deep-sea. Ecology 89:3449-3461

Rosa R, Seibel BA (2008c) Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proc Natl Acad Sci USA 105:20776–20780

Rosa R, Seibel BA (2010a) Slow pace of life of the Antarctic colossal squid. J Mar Biol Assoc 90(7):1375-1378

Rosa R, Seibel BA (2010b) Voyage of the argonauts in the pelagic realm: physiological and behavioural ecology of the rare paper nautilus, Argonauta nouryi. ICES J Mar Sci 67:000–000

Rosa R, Seibel BA (2010c) Metabolic physiology of the Humboldt squid, Dosidicus gigas: Implications for vertical migration in a pronounced oxygen minimum zone. Prog Oceanogr 86:72-80

Rosa R, Trueblood L, Seibel BA (2009) Ecophysiological influence on scaling of aerobic and anaerobic metabolism of pelagic gonatid squids. Physiol Biochem Zool 82:419-429

Savage VM, Gillooly JF, Woodruff WH, West GB, Allen AP, Enquist BJ, Brown JH (2004) The predominance of quarter power scaling in biology. Funct Ecol 18:257-282

Schipp R (1987) General morphological and functional characteristics of the cephalopod circulatory system. An introduction. Cell Mol Life Sci 43:474-477

Schmidt-Nielsen K (1984) Scaling: why is animal size so important? Cambridge University Press, New York

Seibel BA (2007) On the depth and scale of metabolic rate variation: scaling of oxygen consumption rates and enzymatic activity in the Class Cephalopoda (Mollusca). J Exp Biol 210:1-11

Seibel BA, Childress JJ (2000) Metabolism of benthic octopods (Cephalopoda) as a function of habitat depth and oxygen concentration. Deep Sea Res 47:1247-1260

Seibel BA, Dymowska A, Rosenthal J (2007) Metabolic temperature compensation and coevolution of locomotory performance in pteropod molluscs. Integr Comp Biol 47(6):880–891

Webber DM, Aitken JP, O'Dor RK (2000) Costs of locomotion and vertic dynamics of cephalopods and fish. Physiol Biochem Zool 73:651–662

Weibel HR (2002) The pitfalls of power laws. Nature 417:131-132

Weibel ER, Hoppeler H (2005) Exercise-induced maximal metabolic rate scales with muscle aerobic capacity. I Exp Biol 208:1635-1644

Wells MJ (1988) Oxygen extraction and jet propulsion in cephalopods. Can J Zool 68:815-824

Wells MJ (1992) The cephalopod heart: the evolution of a high-performance invertebrate pump. Cell Mol Life Sci 48:800-808

Wells MJ, Smith P (1987) The performance of the octopus circulatory system: a triumph of engineering over design. Cell Mol Life Sci 43:487-499

Wells MJ, Wells J (1982) Ventilatory currents in the mantle of cephalopods. J Exp Biol 99:315-330

Wells MJ, Wells J (1983) The circulatory response to acute hypoxia in Octopus. J Exp Biol 104:59-71

Wells MJ, Wells J (1985) Ventilation frequencies and stroke volumes in acute hypoxia in *Octopus*. J Exp Biol 118:445-448

Wells MJ, Wells J (1986). Blood flow in acute hypoxia in a cephalopod. J Exp Biol 122:345-353

Wells MJ, Wells J (1991) Is *Sepia* really an octopus? In Acta of the 1st International Symposium on the Cuttlefish *Sepia* (ed. E. Boucaud-Camou), pp. 77-92. Caen: Université de Caen Publications

West GB, Brown JH (2004) Life's universal scaling laws. Phys Today 57:36-4

West GB, Brown JH, Enquist BJ (1997) A general model for the origin of allometric scaling laws in biology. Science 276:122-126

White CR, Phillips NF, Seymour RS (2006) The scaling and temperature dependence of vertebrate metabolism. Biol Lett 2:125-127

White CR, Seymour RS (2005) Allometric scaling of mammalian metabolism. J Exp Biol 208:1611-1619