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On the depth and scale of metabolic rate variation: scaling of oxygen consumption rates and enzymatic activity in the Class Cephalopoda (Mollusca)

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Summary

Recent ecological theory depends, for predictive power, on the apparent similarity of metabolic rates within broad taxonomic or functional groups of organisms (e.g. invertebrates or ectotherms). Such metabolic commonality is challenged here, as I demonstrate more than 200-fold variation in metabolic rates independent of body mass and temperature in a single class of animals, the Cephalopoda, over seven orders of magnitude size range. I further demonstrate wide variation in the slopes of metabolic scaling curves. The observed variation in metabolism reflects differential selection among species for locomotory capacity rather than mass or temperature constraints. Such selection is highest among epipelagic squids (Lolignidae and Ommastrephidae) that, as adults, have temperature-corrected metabolic rates higher than mammals of similar size.

Supplementary material available online at
http://jeb.biologists.org/cgi/content/full/210/1/11/DC1

Key words: metabolic scaling, citrate synthase, metabolic theory, deep-sea.

Introduction

The inverse relationship between mass-specific metabolism and body mass, most famously illustrated by the ‘mouse to elephant curve’ for mammals covering six orders of magnitude size range, is among the most established in all of biology (Schmidt-Nielsen, 1984; Calder, 1984; Peters, 1983; Kleiber, 1932; Rubner, 1883; Brody and Proctor, 1932; Heusner, 1991; Hemmingsen, 1960; Zeuthen, 1953; White and Seymour, 2005). Metabolic intensity (i.e. the mass-specific rate of metabolism, B) typically decreases with increasing body mass (M) according to:

$$B = b_0 M^b , \quad (1)$$

where b_0 is a normalization constant independent of mass and the exponent, b, is a scaling coefficient that often falls near quarter-power (b=–0.25) (Savage et al., 2004; Farrell-Gray and Gotelli, 2005). Quarter-power metabolic scaling is viewed by some as a biological law (West and Brown, 2004), and many theories have been postulated to explain the phenomenon [for review and critique (see Glazier, 2005; Agutter and Wheatley, 2004)].

The reported commonality of metabolic scaling patterns across habitats and taxa (Gillooly et al., 2001; Hemmingsen, 1960) has given rise to a ‘metabolic theory of ecology’ (MTE) (Brown et al., 2004) that strives to predict broad ecological and evolutionary trends from rates of energy metabolism in individual organisms. The MTE is purposefully simple, incorporating only mass and temperature, and is thus dependent, for predictive power, on metabolic commonality [i.e. the similarity in normalization constants, b_0, and slopes, b (Eqn 1), across broad taxonomic and functional groups of organisms]. The normalization constant cannot be derived from first principles, but rather, must be fit empirically. Proponents of the MTE acknowledge limited taxon-specific variation in b_0. Gillooly and colleagues (Gillooly et al., 2001) report only sixfold difference between the best-fit metabolic scaling relationships for endotherms and multicellular ectotherms, and 20-fold variation between the lowest unicells and highest mammals.

The MTE is founded on the idea that quarter-power scaling results from universal geometric constraints on the transport of oxygen and fuel with increasing size due to the hierarchical branching networks that characterize many organismal transport systems (West et al., 1997; West et al., 1999). The assumptions of the model have been widely criticized (Chaui-Berlinck, 2006; Suarez et al., 2004; Darveau et al., 2002; Weibel and Hoppeler, 2005; Hulbert and Else, 2005; Porter, 2001; Clarke, 2006; Bokma, 2004; Dodds et al., 2001) and vigorously defended (Gillooly et al., 2006; Brown et al., 2004). Implicit in many of the criticisms is the idea that rates of metabolism reflect organismal energy demand and that constraints on oxygen delivery cannot adequately explain the size-dependence of basal metabolic rate. As such, they have important implications for the MTE and the mechanistic basis for the patterns it describes.

Different normalization constants, found between closely related species living in different environments, different phylogenetic lines, and between athletic and more sedentary species (Biggs, 1977; Childress and Somero, 1990; Reinhold, 1999; Weibel and Hoppeler, 2005; Reich et al., 2006; Makarieva et al., 2005; Seibel and Drazen, in press), are often subtle and appear as noise in the allometric relationships observed over large mass ranges. However, large taxon-specific differences in normalization constant would effectively diminish the generality of the MTE, allowing ecological predictions only for highly specific groups of organisms. Furthermore, species-specific differences in normalization constant will influence interspecific scaling coefficients, regardless of the root cause(s) of scaling relationships.

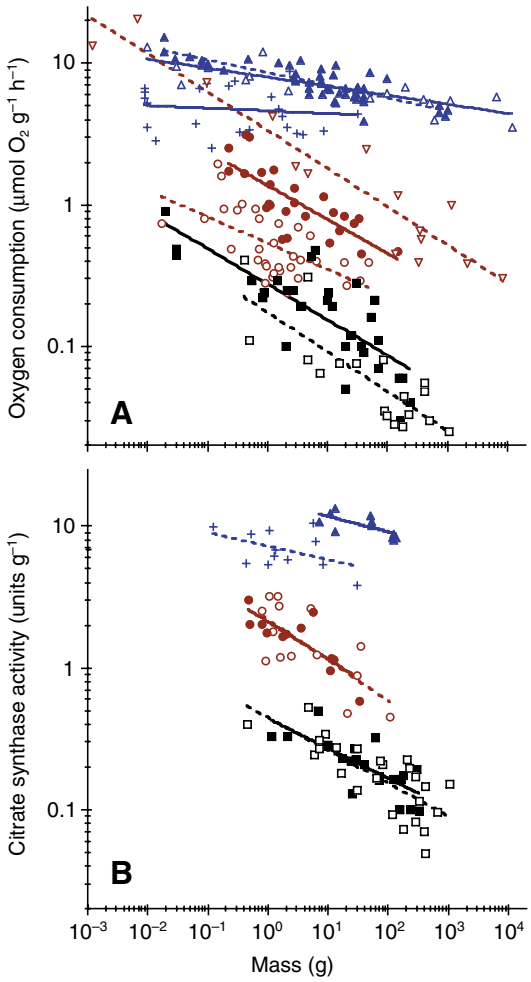

Fig. 1. Effect of body mass on (A) oxygen consumption rates (μmol O_2 g^{-1} h^{-1}; 5°C) and (B) citrate synthase activity (units g^{-1}; 20°C) of cephalopod molluscs. The relationships drawn are for families Loliginidae (filled blue triangles), Ommastrephidae (open blue triangles), Gonatidae (blue crosses), Octopodidae (open brown inverted triangles), Histioteuthidae (brown circles), Cranchidae (open brown circles), Bolitaenidae (black squares), Vampyroteuthidae (white squares). The scaling coefficients for oxygen consumption rates of Loliginid squids are significantly different from benthic Octodidae (ANCOVA, $P<0.05$), and others, and the s.e.m. does not include quarter power (–0.25). Regression equations are presented in Table 1 while sources of data can be found in supplementary material Tables S1–S4.

Here, I analyze routine oxygen consumption rates (Fig. 1A) and activities of citrate synthase (CS; Fig. 1B), a mitochondrial enzyme indicative of the energy demands of steady-state exercise (Moyes, 2003), in eight families within a single class of organisms, the Cephalopoda. Cephalopods are similar to the well-studied mammals in that they occupy an adult size range, from pygmy octopuses to colossal squids, of more than six orders of magnitude. That size range is expanded here to more than seven orders of magnitude by inclusion of ontogenetic metabolic series, from juveniles to adult stages. Furthermore, cephalopods have a closed circulatory system functionally analogous to that of vertebrates and so should, of all invertebrates, most precisely match the conditions required for quarter-power scaling as first outlined by West and colleagues based on the vertebrate circulatory system (West et al., 1997). Cephalopods are found in all marine habitats, from the poles to the tropics, and to the deep ocean trenches below at least 7000 m. They are abundant, commercially important in some cases, and are increasingly dominant components of marine ecosystems (Boyle and Rodhouse, 2005). This is the first comprehensive analysis of metabolic scaling for cephalopods.

Materials and methods

Oxygen consumption and enzymatic activity measurement

All oxygen consumption rates and enzyme activities and their sources are cited in supplemental material online (see supplementary material Tables S1–S4). For inclusion in the present analysis, oxygen consumption rates must have been measured under conditions that minimized, to the extent possible, animal stress, feeding effects (specific dynamic action) and activity levels. Specimens were typically allowed to acclimate to respirometry chambers for periods ranging from 2–12 h. However, activity was directly monitored in only a few cases. For a small subset of these (*Illex illecebrosus*, *Loligo opalescens* and *Lolliguncula brevis*), relationships between oxygen consumption and swimming speed allowed extrapolation to zero activity. In most cases, nothing prevented spontaneous activity within the respirometry chambers and the rates reported here are conservatively referred to as ‘routine’. Activities of citrate synthase, a mitochondrial enzyme indicative of sustained aerobic metabolic capacity) were measured on mantle muscle homogenates at 20°C (well below the denaturation temperature of these enzymes) under substrate-saturating conditions (Seibel et al., 1998; Seibel et al., 2000).

Temperature dependence

All oxygen consumption rates within the families Gonatidae, Cranchidae, Histioteuthidae, Bolitaenidae and Vampyroteuthidae (132 measurements in total), and some

members of the Octopodidae and Ommastrephidae (10 measurements), were measured by the present author at 5°C (Seibel et al., 1997; Seibel and Childress, 2000) (B.A.S., unpublished). All of those species experience 5°C at some point within their daily or ontogenetic distributions. Most individuals in other families (76 measurements in total) were standardized to 5°C assuming a Q_{10} of 2.5. Temperature coefficients reported for some species were unusually high [e.g. Q_{10}=5.6 for *Illex illecebrosus* (DeMont and O'Dor, 1981)] while most others fell near 2.5 (O'Dor and Wells, 1987). Errors in Q_{10} estimation will lead to variation in both slopes and normalization constants of the relationships observed. In the case of normalization constants, such errors are small and could not significantly influence the 200-fold variation observed (more than 100-fold variation is observed just within species measured at 5°C). Scaling coefficients may be much more sensitive to these errors, but arguments presented in the discussion suggest that the present results are not unduly influenced by the method of temperature correction.

The rates of cephalopods are further compared with a variety of other animal taxa, all standardized to 5°C assuming a Q_{10} of 2.5. While one can certainly question the validity of correcting mammalian metabolic rates to 5°C as no mammal will ever experience that temperature, the claims by the MTE of metabolic commonality are based on universal temperature dependence and temperature standardization was necessary to refute that argument. The temperature chosen is of minor significance (although temperature sensitivity may, itself, be temperature dependent in some cases). The finding of exceptionally high metabolic rates among squids is not altered by correcting to 37°C, a temperature only slightly higher than the critical temperature for some species (Pörtner, 2002). Furthermore, the curves for loliginid and ommastrephid squids are above the mammalian curve across the entire size range when adjusted using any Q_{10} value higher than 1.8. Only at unusually low Q_{10} values do the metabolic rates of squids and mammals overlap. Even if no temperature correction is applied to mammals, some overlap between squid (5°C) and mammalian (37°C) metabolic rates is observed.

Statistics and phylogenetic independence

Power regressions were generated using Statview 5.01 (SAS Institute, Cary, NC, USA) and significance of all relationships is at 95% confidence level. Differences in scaling coefficients (i.e. slopes) were assessed by Analysis of Covariance (ANCOVA, Statview 5.01). All oxygen consumption rate data points of a given symbol (Fig. 1) represent individuals within a family. Some families are represented by only one species while others are represented by several. The phylogenetic independence of the data was previously assessed using independent contrasts (Seibel and Carlini, 2001). An analysis of

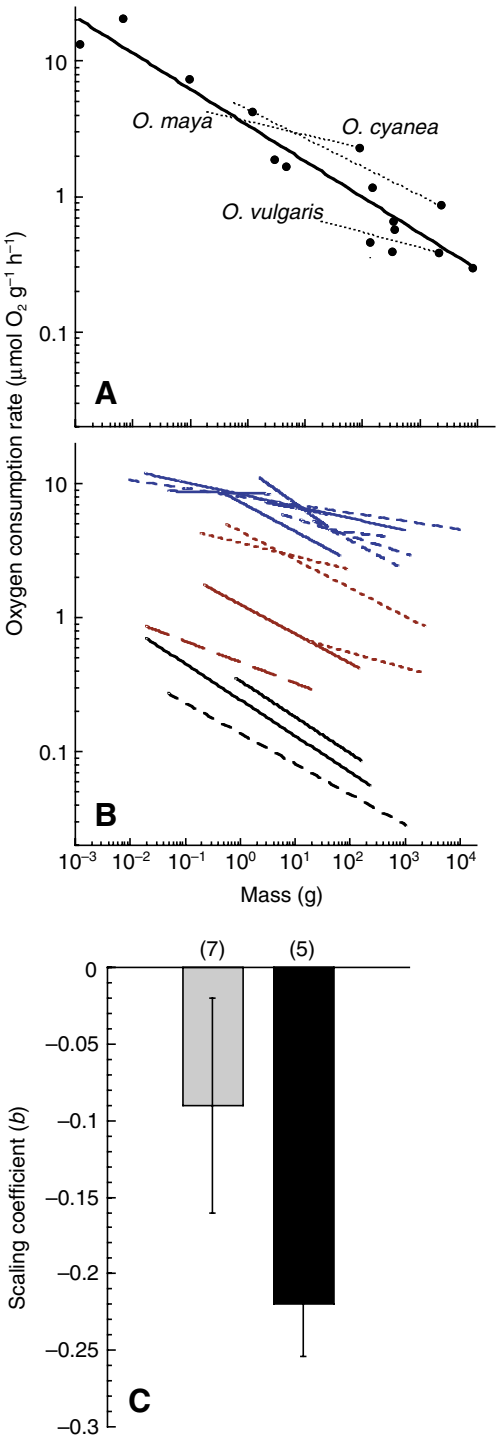

Fig. 2. Intraspecific scaling patterns of cephalopod molluscs. (A) Three published intraspecific scaling curves for benthic octopods are drawn with the interspecific pattern from Fig. 1. (B) Intraspecific scaling relationships are similar to intrafamilial relationships in Fig. 1. Colors and lines represent different families. Loliginidae, solid blue; Ommastrephidae, broken blue; Octopodidae, broken red; Histioteuthidae, solid red; Cranchidae, long broken red; Bolitaenidae, solid black; Vampyroteuthidae, broken black. (C) Intraspecific scaling coefficients (*b* from Eqn 1 in text) are significantly higher (less negative) for epipelagic squids (grey bar; Loliginidae and Ommastrephidae) than for mesopelagic cephalopods (black bar) (*N* in parentheses; values are means ± s.d.). Gonatids are not shown because the relationship between oxygen consumption and body mass was not significant (*b*~0.0). See Table 2 for equations and sources.

higher nodes further demonstrated that most of the variation in cephalopod metabolism is between families within an order, as opposed to species or genera within a family (Seibel and Carlini, 2001). Thus the use of families as the comparative unit allowed greater size range in some cases while not violating assumptions regarding phylogenetic independence of the data. The analysis of intraspecific scaling in Fig. 2 demonstrates a pattern similar to the familial relationships. Phylogeny does not drive the decline in metabolism with depth (Seibel and Carlini, 2001) (Fig. 3).

The use of either whole-animal metabolism or mass-specific metabolism is dependent on preference as well as the question being asked. Obviously a larger organism has more respiring tissue and will consume more oxygen. Thus, a whole-organism scaling curve is, to an extent, a statement of the obvious and provides a false sense of the magnitude of variation. I opted to present mass-specific oxygen consumption rates ($\dot{M}_{O_2}$; μmol O_2 g^{-1} h^{-1}) for this reason and because some phenomena addressed by the MTE, for example rates of DNA base-pair substitution (Gillooly et al., 2005), depend on 'metabolic intensity' rather than whole-organism metabolism. Furthermore, the enzymatic data I present are an inherently mass-specific value measured by grinding a sample of muscle tissue rather than an entire organism. Thus for graphic comparison of the slopes, mass-specific oxygen consumption rates were presented with mass-specific enzymatic activities. Whole-animal metabolic rates lead to the same conclusion and can be calculated simply by multiplying rates by the mass values listed in supplementary material Table S2.

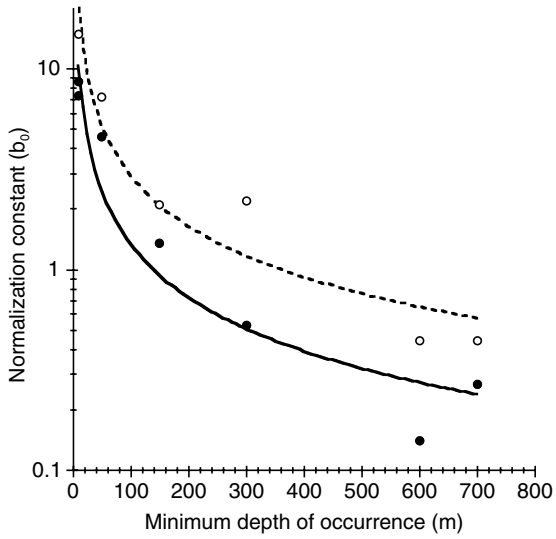

Fig. 3. Minimum depth of occurrence (MDO), defined as the depth below which 90% of the individuals of a given species are captured (Childress, 1995), explains more than 90% of the variation in the normalization constants (b_0) derived from oxygen consumption (solid circles, $b_0=79.1MDO^{-0.9}$; $r^2=0.92$) and citrate synthase activity (open circles, $b_0=141.2MDO^{-0.80}$; $r^2=0.94$) scaling relationships in cephalopod families (Table 1). The family Octopodidae is benthic, allowing similar lifestyles, and hence similar metabolic rates, across a depth gradient as described in the text and elsewhere (Seibel and Childress, 2000). Octopodids are exluded from the analysis presented in this figure. Individuals of some cranchid species can be found occasionally in near-surface waters despite their low metabolic rates, an ability facilitated by their extreme transparency and associated relief from visual predation (Seibel et al., 1997). However, the majority of specimens in this family are collected below 300 m (MDO>300 m).

Results

The influence of body mass, ranging over seven orders of magnitude, on common-temperature rates of routine oxygen consumption and enzymatic activity is presented for cephalopod families (Fig. 1; Table 1), as well as for all individual cephalopods combined (Table 1). As explained in the methods section, the present analysis focuses primarily on family-level relationships as this maximized size range without violating assumptions regarding species phylogenetic independence (Seibel and Carlini, 2001). Most of the variation in metabolism mentioned above is found between families and is directly related to the habitat depth (Fig. 3), which is similar for individual species within each family (Seibel et al., 1997;

Table 1. *Mass-specific oxygen consumption rates and citrate synthase activities as a function of body mass in cephalopod families*

	$\dot{M}_{O_2}=b_0M^b$ (μmol O_2 g^{-1} h^{-1})				CS=b_0M^b (units g^{-1})			
Group	*b*	b_0	r^2	*N*	*b*	b_0	r^2	*N*
Loliginidae	–0.084±0.010	8.20	0.56	51	–0.11±0.035	14.9	0.50	12
Ommastrephidae	–0.077±0.015	7.60	0.60	20	–	–	–	–
Gonatidae	–0.02 (n.s.)	4.57	n.s.	24	–0.10±0.060	7.43	n.s.	13
Octopodidae	–0.27±0.054	3.35	0.90	15	–	–	–	–
Histioteuthidae	–0.24±0.083	1.36	0.58	26	–0.26±0.057	2.10	0.68	13
Cranchidae	–0.19±0.089	0.53	0.31	33	–0.29±0.080	2.2	0.51	16
Bolitaenidae	–0.25±0.072	0.27	0.66	32	–0.21±0.040	0.44	0.58	21
Vampyroteuthidae	–0.23±0.115	0.14	0.56	17	–0.23±0.041	0.44	0.58	20
All Cephalopoda	–0.20±0.038	1.53	0.13	218	–0.42	n.s.	n.s.	–

$\dot{M}_{O_2}$, mass-specific oxygen consumption rate; CS, citrate synthase activity (20°C); *M*, body mass.
Values are means ± s.e.m.; $P>0.05$; n.s., not significant (see supplementary material for full data set).

Seibel et al., 2000). Some variation is found between species within a family, as indicated in Fig. 2; however, additional data is required to determine significance of the differences in most cases.

The variation in normalization constants (b_0; Eqn 1) of cephalopod oxygen consumption and enzymatic activity is explained largely by habitat depth [Fig. 3; $\dot{M}_{O_2}$, $b_0=79.1MDO^{-0.9}$; $r^2=0.92$; CS, $b_0=141.2MDO^{-0.80}$, where MDO=minimum depth of occurrence; $r^2=0.94$; (Seibel et al., 1997; Seibel et al., 2000)]. Individual species within each family are found over similar depth ranges. Normalization constants ranged from 8.2 for ommastrephid squids to 0.14 for the bathypelagic vampire squids. This difference is further enhanced at large sizes due to variation in scaling coefficients. Five of eight cephalopod families analyzed had scaling coefficients (b in Eqn 1) not significantly different from a quarter power. However, each epipelagic squid family (Gonatidae, Loliginidae and Ommastrephidae) had a shallower scaling coefficient that, in the case of Loliginidae, was significantly different from –0.25. Intraspecific scaling slopes are presented in Fig. 2 and Table 2 and most of them are similar to those reported here for familial relationships (Fig. 1; Table 1) (Glazier, 2005; O'Dor and Wells, 1987). The intraspecific scaling coefficients for epipelagic squids are significantly higher (less negative) than for mesopelagic species (Fig. 2C), a pattern consistent with other pelagic animals (Glazier, 2006).

Metabolic rates of cephalopods are compared to a variety of animal taxa living in diverse environments in Fig. 4. Body mass accounted for only 68% of the variation in whole-animal metabolism (13% on a mass-specific basis) within all cephalopods combined. If one considers that additional body mass consumes incrementally more oxygen, a large fraction of the variation in whole-animal metabolism should be explained by body mass. For comparison, recent analyses found that, after temperature adjustment, mass accounts for 94% of the variation in mammalian whole-animal basal metabolic rates (White and Seymour, 2005). Thus, the relatively low correlation coefficients found for combined cephalopod metabolism signifies tremendous interspecific diversity in metabolic rates. All data and their sources are available in supplementary material Tables S1–S4 online.

Discussion

Variation in metabolic rate

In contrast to previous reports of metabolic commonality (Gillooly et al., 2001), Cephalopod metabolism varies between species by as much as 200-fold beyond the effects of mass and temperature (Fig. 1). Furthermore, scaling coefficients of some species deviate significantly from the quarter power relationship that underpins the MTE (Gillooly et al., 2001; Savage et al., 2004; Brown et al., 2004; West et al., 1997; West

Table 2. *Intraspecific scaling coefficients for diverse cephalopods*

Group	Size range (g)	*b*	*N*	Reference
Epipelagic				
Loligo forbesi	0.02–1004.0	–0.09	12	1,2
Lolliguncula brevis	2.31–41.10	–0.09	25	1
	1.7–48.0	–0.24	27	3
Sepioteuthis sepioidea	0.033–68.40	–0.22	70	4
	0.05–3.60	–0.01	35	5
Dosidicus gigas	0.01–12 200	–0.06	10	6
Illex illecebrosus	42.0–443.0	–0.05	–	7
Sthenoteuthis oaulaniensis	22.0–750.0	–0.22	–	8
Sthenoteuthis pteropus	6.0–1300	–0.13	–	8
Mesopelagic				
Histioteuthis heteropsis	0.23–150.0	–0.22	22	9
Liocranchia valdivia	0.02–21.28	–0.19	20	9
Japetella diaphana	0.02–242.17	–0.27	12	9
Japetella heathi	0.84–162.50	–0.27	12	9
Vampyroteuthis infernalis	0.42–1050	–0.23	17	9
Benthic				
Octopus cyanea	0.57–2300	–0.17	28	10
Octopus maya	–	–0.10	–	1
Octopus vulgaris	~20.0–~2000	–0.10	27	11
Octopus vulgaris	250.0–1200.0	–0.28	314	12

b, intraspecific scaling coefficients (Eqn 1).

All data and sources are supplied in supplementary material Table S2.

[1](Segawa and Hanlon, 1988); [2](Boucher-Rodoni and Mangold, 1989); [3](Bartol et al., 2001); [4](Segawa, 1991); [5](Segawa, 1995); [6]L. A. Trueblood, R. Rosa, W. F. Gilly and B.A.S., unpublished; [7](DeMont and O'Dor, 1984); [8](reviewed by Zuyev et al., 2002); [9](Seibel et al., 1997); [10](Maginniss and Wells, 1969); [11](Katsanevakis et al., 2005); [12](Wells et al., 1983).

et al., 1999) (Fig. 1; Tables 1, 2). Mass, while an important metabolic determinant *within* many individual species (Fig. 2), is a relatively minor determinant of routine metabolism among all cephalopods at a common temperature. As described in detail below, large taxon-specific differences in normalization constant within Cephalopod molluscs (Fig. 1), and more broadly (e.g. fishes), reflect important interspecific differences in energy demand as dictated by the particular ecological niche inhabited by a species (e.g. habitat depth and foraging mode, Fig. 3). The observed metabolic variation is not restricted to cephalopods (Fig. 4), a fact that calls into question the generality of metabolic scaling and the application of scaling to macroecological theory.

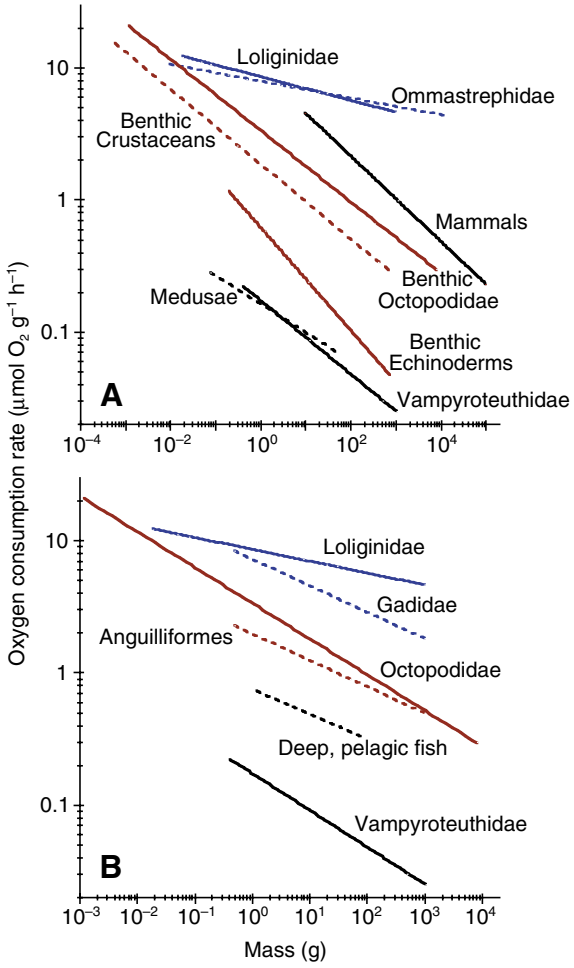

Fig. 4. Comparisons of cephalopod oxygen consumption rates with those from other distantly related taxa (adjusted, as necessary, to 5°C). (A) Epipelagic loliginid and ommastrephid squids are compared with deep-sea vampyroteuthid squids, mammals (White et al., 2006), medusae (pelagic cnidarians) (Thuesen and Childress, 1994), and benthic crustaceans and echinoderms (Seibel and Drazen, in press). (B) Cephalopods are compared with pelagic fishes (broken lines) including gadids (cod), benthic anguilliform eels (Clarke and Johnston, 1999), and a deep-sea pelagic anglerfish, *Melanocetes johnsoni* (Cowles and Childress, 1995). Statistics and data sources are given in Table 1 and supplementary material Tables S1, S2 and S4.

Ecological basis of metabolic variation

The majority of metabolic variation observed here stems from differential selection for muscular energy demand [i.e. locomotory capacity (Seibel et al., 1997; Seibel et al., 1998; Seibel et al., 2000)]. Locomotory capacity is an important determinant of animal metabolism in any environment, but its influence is enhanced within the expansive pelagic biosphere by the depth-related gradient in light available for predator–prey interactions (Childress, 1995; Seibel and Drazen, in press). Shallow-living squids (referred to hereafter as 'epipelagic') spend at least some portion of their day in active pursuit of prey through surface waters. In nature, such squids swim continuously by jet propulsion at low efficiencies (O'Dor and Webber, 1986) and have consequently evolved among the highest temperature-corrected metabolic rates of any organisms, including heterothermic fishes, flighted insects and mammals (Fig. 4) (O'Dor and Webber, 1986; Shulman et al., 2002; Clarke and Johnston, 1999; Reinhold, 1999; O'Dor and Webber, 1986). Interestingly, metabolism in coastal loliginids is indistinguishable from the more oceanic ommastrephids despite several published reports to the contrary (Fig. 1A). In contrast, demand for locomotion among deep-living (meso- and bathypelagic) sit-and-wait predators, as indicated by physiological proxies as well as direct submersible and shipboard observations, is greatly diminished (Seibel and Drazen, in press; Seibel et al., 2000). Such species swim sluggishly, but with greater efficiency than shallower-living species (Seibel et al., 1997; Seibel et al., 1998; Seibel et al., 2000).

Habitat depth and visual predator–prey interactions

The present analysis confirms that bathypelagic cephalopods have routine metabolic rates and enzymatic activities up to 200-fold lower than their shallow-living relatives (Fig. 1). Size-corrected metabolism and citrate synthase activity (normalization constants from relationships in Fig. 1A,B; Table 1) in cephalopods are strongly and inversely related to the minimum habitat depth occupied by a family (Fig. 3) (Seibel et al., 1997; Seibel et al., 2000). That these depth trends are independent of mass and temperature is made clear by the present analysis. The close correspondence between scaling relationships for oxygen consumption rates and enzymatic activities argues strongly that these results are not artifactual (Fig. 1; Table 1). However, the extent to which metabolic rates decline with depth depends on the size chosen for normalization because of the divergent scaling relationships of deep- and shallow-living species.

Strong depth-related trends in metabolism and enzymatic activity have been reported only for visually orienting pelagic predators, such as fishes, crustaceans and cephalopods (Childress, 1995; Seibel et al., 1997; Torres and Somero, 1988). More than 30 years of careful comparative study demonstrates these trends to be independent of surface productivity (a proxy for food availability), oxygen content and phylogeny (for reviews, see Childress and Seibel, 1998; Childress, 1995; Thuesen et al., 1998; Seibel and Carlini, 2001; Seibel and

Drazen, in press). The decline in metabolism with depth is explained, not by environmental constraint at depth, but by strong selection for high locomotory capacity in well-lit surface waters and a relaxation of that selection with light-limitation at depth [i.e. the 'visual interactions hypothesis' (Childress, 1995; Seibel and Drazen, in press)].

Visible light decreases linearly with depth and is absent below 1000 m. According to the 'visual interactions hypothesis', the distances over which visually orienting predators and prey detect each other, and the distances they must swim to catch or avoid one another, are substantially reduced in the deep pelagic biome. Consequently, the requirement for high metabolic rates and, as indicated by mitochondrial enzyme activity in locomotory muscles, locomotory capacity, is similarly reduced (Fig. 1B) (Seibel et al., 2000). The depth-related decline is especially pronounced in cephalopods due to the differences in locomotory efficiency and buoyancy between deep- and shallow-living species (Seibel et al., 1997; Seibel et al., 2000; Seibel et al., 2004) and to the divergent scaling relationships demonstrated here. Reduced demand for locomotion is generally accompanied by reduced protein, increased water and lower mitochondrial abundance in locomotory muscles, all of which contribute to reduced resting or routine metabolic rates (Childress, 1995; Seibel et al., 2004).

The visual interactions hypothesis is supported by the lack of depth-related trends in metabolism for non-visual pelagic taxa [e.g. medusae, chaetognaths or copepods (Thuesen and Childress, 1994; Thuesen and Childress, 1994; Thuesen et al., 1998)], and in benthic visual taxa that have greater opportunities for crypsis and refuge from predation (Childress et al., 1990; Seibel and Childress, 2000). All species within each of the pelagic cephalopod families analyzed here are found at similar depths (e.g. all loliginids are shallow-living while all bolitaenids are deep-living) but this is not true of the benthic Octopodidae. However, benthic octopods, as well as other benthic groups, appear to have temperature-corrected metabolic rates that do not vary significantly with habitat depth (Fig. 1A). The similarity in metabolic rates within benthic groups of organisms is a reflection of the similarity of lifestyles among benthic species (Childress, 1995; Seibel and Childress, 2000; Seibel and Drazen, in press). While benthic animals can burrow in the sediment and hide in crevices to avoid detection by predators, pelagic species lack such refuge.

Metabolic state and the meaning of enzymatic activities

Implicit in the visual interactions hypothesis is the idea that the measured routine or resting metabolic rates reflect the metabolic and locomotory demands on these animals in nature. Such 'field' metabolic rates are obviously intermediate between resting and maximum rates of metabolism (O'Dor, 2002; O'Dor et al., 1994). Studies have documented a close relationship, despite divergent scaling relationships in some cases (Weibel and Hoppeler, 2005), between resting and maximum aerobic metabolic rate (Reinhold, 1999). The nature of this linkage is not fully understood but may relate to added maintenance costs for machinery that supports elevated locomotory activity as well as evolutionary trade-offs between resting costs and scope for activity (Clarke, 2006; Reinhold, 1999; Seibel and Drazen, in press).

Citrate synthase activities typically correlate with the metabolic capacity required for sustained exercise [e.g. active or maximum, rather than resting, metabolic rates (Moyes, 2003; Weibel and Hoppeler, 2005)]. However, at least in a broad interspecific comparison, such as that presented here, a correlation between enzymatic activity and routine rates is not surprising and reflects divergent locomotory capacity between deep- and shallow-living species as discussed above. The apparent correlation between scaling coefficients for enzymatic activity and routine oxygen consumption rate are intriguing and require further study. Scaling of maximum metabolic rate has not been addressed systematically in cephalopods but the few available data suggest a positive mass-specific scaling coefficient. Smaller species of loliginid squids appear to have limited aerobic scope compared to larger species (e.g. Bartol et al., 2001; Webber and O'Dor, 1986; Finke et al., 1996).

Scaling coefficients

The present data do not support the existence of a universal scaling coefficient (Table 1). Three of eight families have scaling coefficients significantly different from quarter power. The shallower slopes all belong to families of epipelagic squids and are consistent with the few intraspecific literature values available (Table 2; Fig. 2B). A significant difference was found between intraspecific scaling coefficients of epipelagic and mesopelagic cephalopods (Fig. 2C). O'Dor and colleagues (DeMont and O'Dor, 1984; Webber and O'Dor, 1986; O'Dor and Wells, 1987) controlled activity and body size independently in *Illex illecebrosus* and found that mass-specific metabolism was not dependent on body mass, at least over the limited size range available. Similar results have been reported previously for ommastrephid (Zuyev et al., 2002), onychoteuthid (reviewed in O'Dor and Wells, 1987) and loliginid (Segawa, 1995; Segawa and Hanlon, 1988; Wells et al., 1988) squids. A few studies report an intraspecific exponent near quarter-power for epipelagic squid species (Table 2) (Segawa, 1991; Bartol et al., 2001) but conflicting studies exists for the same species. Combining studies to enhance the intraspecific size range reveals a shallow scaling coefficient for *Loligo forbesi* (b=–0.09; Loliginidae; Table 2) and preliminary data suggest a shallow slope for *Dosidicus gigas* over six orders of magnitude size range (b=–0.06, Table 2).

Interestingly, the intra- and interspecific scaling relationship within the Octopodidae varied. While the present interspecific study demonstrates scaling near a quarter-power for benthic octopods, three independent studies report relatively shallow intraspecific scaling coefficients for this group (Maginnis and Wells, 1969; Segawa and Hanlon, 1988; Katsanevakis et al., 2005). Only one intraspecific study (Wells et al., 1983) showed quarter-power scaling (Fig. 2A; Table 2). Obviously the relationship between intra- and interspecific metabolic scaling remains an open and intriguing question that hinges on the

trade-off between required size range, sample size, and the similarity of normalization constants (b_0) and slopes (b) for the species included (see Table 2). The slope of an interspecific relationship may be significantly altered by species-specific differences in scaling parameters. Thus, the method used must depend on the precision required for the question between addressed.

While maximum metabolic rates in mammals (Weibel and Hoppeler, 2005) scale with a shallow slope similar to that reported here for epipelagic squids, it is important to point out that the present squid data do not reflect high levels of activity during measurement. The rates presented here are termed 'routine' because spontaneous activity within the respirometry chambers was not controlled in most cases, but *all rates were measured under conditions that minimized activity levels to the extent possible.* For example, measurements for which activity was not specifically controlled were performed on animals that were acclimated for several hours, post-absorptive, and held in darkened respirometry chambers. Maximum, active or field metabolic rates have only been measured for some species in the families Loliginidae and Ommastrephidae (O'Dor, 2002) and they are substantially higher than the rates used in the present analysis.

Shallow scaling relationships are also not an artifact of temperature correction. All rates presented here were measured at 5°C with the exception of those in the families Loliginidae, Ommastrephidae and some benthic Octopodidae. Metabolism in those families was adjusted to 5°C, assuming a Q_{10} of 2.5. While published Q_{10} values for cephalopods vary widely, most fall near this value (O'Dor and Wells, 1987). Sufficient data exists for the family Loliginidae and Ommastrephidae, combined, to analyze scaling at a common measurement temperature of 13±1°C. That analysis also revealed a shallow slope over a mass range of more than six orders of magnitude (b=–0.15, N=8). Furthermore, as stated above, published mass-specific metabolic scaling coefficients for loliginid and ommastrephid squids are generally greater (less negative) than –0.25 (Fig. 2B,C; Table 2). Lastly, the close correspondence between enzymatic activities (all measured at 20°C) and oxygen consumption rates (measured or adjusted to 5°C), in both b_0 and b, provides assurance that the errors in temperature correction are not substantially influencing the observed relationships (Fig. 1).

Despite the tremendous numbers of variables that could work synergistically to cause the observed scaling relationship for any given species (Suarez et al., 2004), hypotheses put forward to explain the phenomenon of metabolic scaling generally fall into only a few categories (see Glazier, 2005; Agutter and Wheatley, 2004). Most hypotheses reflect the slower rate of increase of effective surface area or cross section of a solid as its mass increases (Childress and Somero, 1990). Many hypotheses, including the recent contribution upon which the MTE is founded (e.g. West et al., 1997; West et al., 1999; Banavar et al., 1999), suggest that geometric scaling rules impose constraints on the design of animals such that some surface (be it internal or external)-limited supply or removal processes (e.g. gas exchange or digestion) increases more slowly than does mass and that metabolism necessarily follows. Alternatively, Childress and Somero argue (Childress and Somero, 1990) that the usual negative allometry of basal aerobic metabolism is due to increased (geometric) opportunities for energy savings in larger animals as a result of reduced costs at large sizes for such processes as thermoregulation in mammals, ion regulation in fishes (Childress and Somero, 1990), or cost of transport in mobile species (Suarez et al., 2004; Glazier, 2006). Cephalopods shed unique light on this ongoing debate but ultimately provide no resolution because several plausible, non-exclusive, hypotheses can be formulated that explain, equally well, the near-isometric metabolic scaling observed in epipelagic squids.

Cutaneous respiration and exchange surfaces

Despite remarkable ecological and physiological convergence with marine vertebrates (Seibel and Drazen, in press; O'Dor and Webber, 1986), epipelagic squids exhibit a few key differences in form and function that, in combination, may alleviate the hypothesized constraints or deny squids the hypothesized opportunities for energy savings associated with the scaling of exchange surfaces. The oxygen-carrying capacity of the cephalopod circulatory system is limited, relative to vertebrates of similar aerobic capacity, requiring that they make maximal use of all blood-borne oxygen, even at rest (Pörtner, 2002; Finke et al., 1996). However, carbon dioxide production is in excess of the oxygen capacity of the blood suggesting that they acquire additional oxygen, as much as 60% of demand, across the skin (Pörtner, 2002). Diffusion distances typically increase with animal size, presumably diminishing the utility of cutaneous oxygen uptake in large animals. However, the body of epipelagic squids is effectively a hollow tube, with internal and external surfaces inherently well suited for cutaneous oxygen uptake. As such squids grow, their mantle diameter increases faster than thickness. As a result, total surface area relative to volume ($SA^{1/2}$:$V^{1/3}$) actually increases with size (O'Dor and Hoar, 2000). Blood vessel density is relatively low throughout the mantle while mitochondria are most abundant along the internal and external surfaces (Bone et al., 1981; Mommsen et al., 1981), an arrangement that maximizes the P_{O_2} gradient across the skin and ensures maximal use of diffused oxygen.

Thus, if exchange surfaces are critical determinants of metabolic scaling, metabolism in epipelagic (tube-shaped) squids are expected to diverge from other cephalopod groups with increasing size. In support of this hypothesis I show that scaling of mass-specific metabolism and citrate synthase activity in epipelagic squids (Loliginidae, Ommastrephidae and Gonatidae) approaches mass-independence (b<–0.10) while metabolism in other cephalopod groups scales near a quarter-power (Fig. 2C). Other cephalopods are also dependent to varying extents on cutaneous respiration [e.g. *Octopus vulgaris* (Madan and Wells, 1996)]. However, the ratio of surface to volume does not increase with size in other species (i.e.

$SA^{1/2}$:$V^{1/3}$ is constant across the size range). The comparison between squids and octopods should be viewed with some caution because, although the present analysis shows quarter-power scaling in the benthic family Octopodidae, three (of four) intraspecific metabolic scaling studies for this group found shallow scaling coefficients, similar to the epipelagic squids as discussed above (Fig. 2A). The near-isometric metabolic scaling in some pelagic taxa [e.g. ctenophores and salps (Glazier, 2005; Glazier, 2006)] may reflect, as I have suggested above for squids, near isometric scaling of surface to volume ratios in conjunction with reliance on cutaneous respiration (cf. Thuesen et al., 2005).

Cost of transport

One recent hypothesis suggests that size-related increases in the energy costs of swimming or of rapid rates of growth and reproduction in response to high levels of mortality (predation) in open water may lead to scaling coefficients that approach isometry in epipelagic animals (Glazier, 2005; Glazier, 2006). Glazier notes several phyla of pelagic animals within which metabolism scales isometrically, while quarter-power scaling is more common in benthic species of these same phyla (Glazier, 2006). Four families of pelagic cephalopods examined here scale near quarter-power, but all are deep-living and presumably experience lower levels of predation. In support of Glazier's hypothesis, the fast-growing, high-capacity species scale nearly isometrically. Benthic octopods, with an exponent near quarter-power, also appear to support Glazier's argument (but as noted above, the intraspecific scaling coefficients differ). However, many other pelagic taxa scale near quarter-power suggesting that other factors must also be at work (e.g. Seibel and Dierssen, 2003; Thuesen and Childress, 1994) [but for comprehensive survey, see Glazier (Glazier, 2005)].

Several authors have postulated a relationship between metabolic scaling and locomotory costs. Stride or fin stroke frequency is directly proportional to power output or metabolic cost, and necessarily declines with size (Suarez, 1996; Suarez et al., 2004; Glazier, 2005; Seibel et al., 1998; Schmidt-Nielsen, 1984; Bejan and Marden, 2006). The observed scaling patterns may, thus, reflect the cost-of-transport (COT), which decreases rapidly with size in mammals, birds and fishes (Suarez et al., 2004; Childress and Somero, 1990), but not in epipelagic squids. Limited data suggests that cost of transport scales differently for jet propulsion ($M^{-0.2}$) and fin swimming ($M^{-0.3}$), such that jetting is more efficient than fin swimming at small, but not large, sizes (O'Dor and Webber, 1986; Seibel et al., 1998; Thompson and Kier, 2001). The divergent scaling coefficients for epipelagic squids and fishes suggest that COT may play a role in metabolic scaling.

Conclusions

Metabolic rates in the Cephalopoda, a single class of organisms, span nearly the entire scale of known metabolic variation in organisms (Makareiva et al., 2005; Suarez, 1996; Reinhold, 1999; Seibel and Drazen, in press). To put the metabolic variation in perspective, a deep-living vampire squid (*Vampyroteuthis infernalis*) weighing just 1 g has the same mass-specific metabolic rate as an elephant (~10^6 g; extrapolation of the curve in Fig. 3) while an epipelagic squid weighing 10 kg has the same mass-specific metabolic rate as a mouse (~10 g; Fig. 3). Only brief behavioral observations are required to demonstrate qualitatively that deep- and shallow-living pelagic cephalopod species have a vastly different mode and pace of life despite overlapping body mass and temperature ranges. Nevertheless, proponents of the MTE (Brown et al., 2004; Gillooly et al., 2001) have overstated the influence of body size and temperature on metabolic rate to the exclusion of such obvious differences in lifestyle, ecology and evolution.

The limited survey of available metabolic data from which reports of metabolic commonality are derived and on which the MTE is founded, certainly contributes to a lack of appreciation for ecological influences. For example, the invertebrate data cited by Gillooly et al. include only 25 measurements of 15 species (Gillooly et al., 2001). While they represent diverse phyla, all included species have similar lifestyles that involve crawling on, or burrowing in, the ground or sediment. There are no flying, swimming or floating representatives. It is therefore not too surprising that the data approximate a single scaling relationship once corrected for temperature differences. Seibel and Drazen found that the variation in normalization constants between benthic groups within a phylum is not nearly as pronounced as that for pelagic groups in those same phyla (Seibel and Drazen, in press). There is, of course, substantial variation within and between groups of benthic organisms, but normalization constants of benthic groups cluster more closely than do pelagic groups reflecting a more limited range of activity levels on the benthos regardless of depth (Fig. 4) (Seibel and Childress, 2000).

The diversity of metabolic rates within the Cephalopoda should not be viewed as exceptional. The pelagic biosphere is the largest ecosystem on the planet (Robison, 2004) and the metabolic rates of its inhabitants, both invertebrates and vertebrates, cannot be adequately described, nor even approximated, by a single allometric relationship that incorporates only mass and temperature (Figs 1, 2). For example, the ecologically important and abundant cnidarians and ctenophores push the envelope with the bathypelagic cephalopods at the lower, while the tunas, epipelagic sharks and flighted insects join epipelagic squids at the upper, end of the metabolic spectrum (Fig. 4). Substantial variation in normalization constants has been reported within fishes, crustaceans, annelids, mollusks (Fig. 4) (Seibel and Drazen, in press), mammals (Weibel and Hoppeler, 2005), insects (Reinhold, 1999), plants (Reich et al., 2006) and unicells (Makareiva et al., 2005). Wide variation in intra- and inter-specific scaling coefficients (b; Eqn 1) has also been reported (Glazier, 2005; Glazier, 2006).

Thus, I suggest that mass, while an important metabolic determinant within appropriately constrained phylogenetic or

functional groups, is not an especially useful predictor of metabolism within such broad groups of organisms as ‘invertebrates’, ‘vertebrates’ or ‘ectotherms’. Moreover, there is no valid reason for separating vertebrates from invertebrates or ectotherms from endotherms in metabolic rate models, given that all fish and mammalian rates fall within the range of invertebrate data (Fig. 4). The appropriate taxonomic level of analysis depends on the question being asked and thus, the precision with which metabolic rates must be modeled. An ecological model that requires independent determination of scaling coefficients and normalization constants for each species is of limited value. My analysis reveals the predictive limitation of the MTE by demonstrating that metabolic scaling relationships (and their hypothesized mechanistic basis) are not universal and that mass is not the primary determinant of metabolic intensity. The divergent lifestyles of deep- and shallow-living pelagic cephalopods provide an ecological ‘signal’ for metabolism that clearly emerges from the allometric ‘noise’ and demonstrates a dominant role of ecology in determining metabolic rates.

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Table S1. *Oxygen consumption rate mass-scaling relationships for cephalopod families*

Group	Mass range (g)	b[a]	b_0	N	r^2	Source
Loliginidae	0.019–1004	–0.084±0.010	8.2	51	0.56	Table 2
Ommastrephidae	0.01–12 200	–0.078±0.015	7.6	20	0.60	Table 2
Gonatidae	0.009–31.28	–0.02 (n.s.)	n.s.	24	n.s.	Table 2
Octopodidae	0.001–8200	–0.27±0.054	3.35	15	0.90	Table 2
Histioteuthidae	0.23–150	–0.24±0.083	1.36	26	0.58	Table 2
Cranchidae	0.017–47.9	–0.19±0.089	0.53	33	0.31	Table 2
Bolitaenidae	0.016–242	–0.25±0.072	0.27	32	0.66	Table 2
Vampyroteuthidae	0.41–1050	–0.23±0.115	0.14	17	0.56	Table 2
All Cephalopoda	0.001–12 200	–0.20	1.53	218	0.13	Table 2
Gadidae (pelagic fish)	–	–0.20	7.20	–	–	29
Anguilliformes (benthic eels)	–	–0.20	2.00	–	–	29
Melanocetes johnsoni (bathypelagic fish)	–	–0.19	0.75	7	0.37	30
Medusae (jellyfish)	–	–0.22	0.16	63	0.20	32
Mammals	–	–0.32	9.47	–	–	33
Benthic Crustaceans	–	–0.28	1.90	58	0.88	34
Benthic Echinoderms	–	–0.39	0.61	40	0.52	34

Mass-specific rate=$b_0 M^b$.

[a]Values are means ± s.e.m.; n.s.=not significant. All other relationships are significant at $P<0.05$.

Table S2. *Oxygen consumption rates and body masses of all individual cephalopods used in Fig. 1*

Family: Species	Mass (g)	$\dot{M}_{O_2}$ at 5°C (μmol O_2 g^{-1} h^{-1})	Reference[a]
Loliginidae			
Sepioteuthis lessoniana	0.05	8.7200	1
	0.19	8.6100	1
	0.48	8.5200	1
	1.53	8.4280	1
	3.60	8.3400	1
	0.43	8.76	35
	68.5	2.87	35
Lolliguncula brevis	2.3	9.73	2
	7.5	8.15	2
	12.25	8.23	2
	15.75	6.08	2
	27.70	5.76	2
	41.10	3.86	2
	13.5	9.65	3
	8.02	9.15	4
	40.0	5.75	5
	8.2	5.98	5
	30	5.98	5
	8	7.18	5
	8	7.54	5
	5	5.98	5
	3	8.38	5
	3	7.42	5
	5	7.30	5
	5	6.58	5
	10	6.58	5
	11	6.46	5
	13	5.39	5
	14	5.27	5
	20	5.98	5
	20	6.58	5
	30	6.46	5
	32	6.82	5
	40	5.39	5
	40	6.58	5
Loligo pealei	50	8.66	6
	100	7.70	6
Loligo forbesi	0.019	15.26	5
	0.019	12.08	5
	0.029	10.53	5
	0.029	10.75	5
	0.08	10.57	5
	0.08	9.73	5
	0.10	9.23	5
	0.10	9.89	5
	738	5.06	7
	745	4.46	7
	1004	4.62	7
	937	4.18	7
Loligo opalescens	41.0	5.66	8
	30.0	5.53	9
Ommastrephidae			
Illex illecebrosus	100	6.16	10
	443	4.00	11
	42	4.50	11
	400	5.60	12

	500	5.27	12
	200	6.76	12
Dosidicus gigas	12200	3.50	24
	6600	5.78	24
	0.50	8.15	13
	0.43	7.55	13
	1100	5.48	24
	0.037	7.10	24
	0.010	13.12	24
	0.031	9.54	24
	2.20	6.41	24
	0.85	12.07	24
Sthenoteuthis oualaniensis	22.0	5.24	36, 37
	750	2.41	36. 37
Sthenoteuthis pteropus	6.0	5.83	36, 37
	1300	2.9	36, 37
Gonatidae			
Gonatus onyx	1.560	4.694	13
	0.074	6.608	13
	8.520	3.286	13
	0.510	3.395	13
	1.750	4.231	13
	1.171	5.775	13
	0.096	7.184	13
	0.227	6.859	13
	0.284	7.45	13
	0.065	3.72	13
	0.010	3.49	13
	0.010	5.25	13
	0.009	6.65	13
	0.009	6.30	13
	0.009	5.69	13
	0.014	2.83	13
	0.117	2.51	13
	0.340	3.25	13
	2.31	8.79	14, 25
Gonatus pyros	2.500	7.600	14
	3.120	3.200	14
	2.170	3.530	14
	3.840	3.152	14
	31.280	4.417	14, 25
Octopodidae			
Octopus bimaculoides	360	0.57	15
Octopus briareus	345.5	0.653	19
Octopus californicus	330	0.39	15
Octopus cyanea	1150	0.99	20
Octopus dofleini	8200	0.30	21
Octopus sp. (unid. juv)	0.0068	20.35	18
Octopus maya	45.1	2.45	5
Octopus micropyrsus	4.65	1.65	15
Octopus rubescens (juv)	0.096	7.4	13
Octopus sp. (unid.juv)	0.0012	13.4	13
Octopus vulgaris	2160	0.38	22
Ocythoe tuberculata (male)	1.21	4.17	14
Paraledone characoti	136.7	0.45	23
Bathypolypus articus	3.0	1.88	17
Eledone cirrhosa	150	1.17	23
Histioteuthidae			
Histioteuthis hoylei	1.27	1.77	14
	0.44	3.12	13
	0.41	1.66	14

	8.51	1.133	14
Histioteuthis heteropsis	4.25	0.83	16
	2.85	1.04	14
	2.85	1.32	14
	1.09	1.00	14
	2.07	0.90	14
	27.10	0.73	14
	15.87	0.65	14
	0.23	2.50	14
	36.98	0.45	14
	0.97	0.95	14
	1.09	1.39	14
	1.03	1.02	14
	0.23	1.72	14
	2.09	0.58	14
	0.80	1.69	14
	0.51	2.98	14
	18.92	0.83	14
	1.79	0.57	14
	33.97	0.79	14
	10.94	0.54	14
	12.68	0.88	14
	150.00	0.47	13
Cranchidae			
Liocranchia valdivia	2.47	0.36	14
	21.28	0.27	14
	0.92	0.28	14
	1.57	0.34	14
	3.27	0.70	14
	0.71	0.49	14
	0.17	1.61	14
	0.25	0.49	14
	2.69	0.43	14
	0.90	0.38	14
	1.30	0.54	14
	1.50	0.74	14
	1.23	0.31	14
	0.96	0.37	14
	0.68	0.41	14
	0.017	0.74	14
	1.25	0.33	14
	19.00	0.39	14
	0.09	0.79	14
	3.27	0.30	14
Helicocranchia pfeferi	0.19	0.94	14
	0.31	0.91	14
	0.15	1.94	14
	0.38	1.01	14
	0.63	0.93	14
	0.71	0.79	14
	3.60	0.41	14
Cranchia scabra	6.39	0.40	14
	35.39	0.29	14
Galiteuthis phyllura	5.19	0.61	14
Liocranchia pacificus	2.12	0.24	14
	1.52	0.36	14
Megalocranchia sp.	47.9	0.39	14
Bolitaenidae			
Eledonella pygmaea	20.2	0.05	14
	40.0	0.09	14
	5.40	0.43	14
	2.03	0.10	14

	3.4900	0.19	14
	11.800	0.19	14
	60.821	0.21	14
	3.7300	0.19	14
Japetella heathi	6.3200	0.48	14
	10.427	0.24	14
	53.040	0.16	14
	71.776	0.11	14
	30.586	0.28	14
	20.330	0.10	14
	9.7460	0.21	14
	0.84000	0.22	14
	25.504	0.12	14
	162.50	0.03	14
	0.87	0.24	14
	1.49	0.29	14
Japetella diaphana	36.0	0.10	14
	24.09	0.12	14
	70.85	0.07	14
	0.56	0.29	14
	242.17	0.04	14
	2.12	0.25	14
	153.25	0.06	14
	182.07	0.06	14
	0.03	0.49	14
	0.03	0.44	14
	2.69	0.25	14
	0.02	0.89	14
Vampyroteuthidae			
Vampyroteuthis infernalis	15.9	0.075	14
	225.0	0.033	14
	425.0	0.048	14
	30.0	0.075	14
	7.32	0.065	14
	185.20	0.044	14
	510.0	0.030	14
	425.0	0.055	14
	87.10	0.035	14
	100.0	0.032	14
	83.7	0.080	14
	180.0	0.027	14
	0.41	0.41	14
	4.74	0.080	14
	4.74	0.31	14
	0.50	0.11	14
	1050.0	0.025	14
	130.6	0.028	14

[a]For references, see Data Sources (Table S4).

Table S3. *Citrate synthase activity mass scaling relationships for cephalopod families at 20°C*

Group	Size range (g)	*b*	b_0	*N*	r^2	Reference[a]
Loliginidae						
Loligo pealei	7.0–135.0	–0.11±0.035	14.9	12	0.45	24
Gonatidae						
Gonatus onyx	0.12–31.08	–0.10±0.06	7.2	13	n.s.	25, 26
Histioteuthidae						
Histioteuthis heteropsis	0.46–33.97	–0.26±0.057	2.1	13	0.58	28
Cranchidae						
Liocranchia valdivia	0.78–106.0	–0.29±0.08	2.2	16	0.40	28
Bolitaenidae						
Japetella diaphana	1.14–300.0	–0.21±0.04	0.44	21	0.52	28
Japetella heathi						
Vampyroteuthidae						
Vampyroteuthis infernalis	0.45–1050.0	–0.23±0.041	0.44	20	0.61	27
All Cephalopoda	–0.12–1050	–0.42 (n.s.)	2.80	95	0.06	–

Activity=$b_0 M^b$.

Values for *b* are ± s.e.m.; n.s.=not significant.

[a]For references, see Data Sources (Table S4).

Table S4. *Data sources*

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