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Slow swimming, fast strikes: effects of feeding behavior on scaling of anaerobic metabolism in epipelagic squid

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ABSTRACT
Many pelagic fishes engage prey at high speeds supported by high metabolic rates and anaerobic metabolic capacity. Epipelagic squids are reported to have among the highest metabolic rates in the oceans as a result of demanding foraging strategies and the use of jet propulsion, which is inherently inefficient. This study examined enzymatic proxies of anaerobic metabolism in two species of pelagic squid, *Dosidicus gigas* and *Doryteuthis pealeii* (Lesueur 1821), over a size range of six orders of magnitude. We hypothesized that activity of the anaerobically poised enzymes would be high and increase with size as in ecologically similar fishes. In contrast, we demonstrate that anaerobic metabolic capacity in these organisms scales negatively with body mass. We explored several cephalopod-specific traits, such as the use of tentacles to capture prey, body morphology and reduced relative prey size of adult squids, that may create a diminished reliance on anaerobically fueled burst activity during prey capture in large animals.

KEY WORDS: Tentacles, Glycolitic scaling, Metabolic capacity

INTRODUCTION
Maximal speed is largely accepted as playing a key role in predator–prey interactions (Hertz et al., 1988). Epipelagic fishes are characterized by high metabolic rates and size-independent burst speeds during prey capture of ~10 body lengths s^{-1} (Webb, 1977; Wu, 1977). These high relative speeds are presumably required across the size range to effectively capture prey because the distance over which predators and prey detect each other increases with body mass (M). Thus, in the shallow pelagic environment where there is little refuge from predation (Johnsen, 2001; Robison, 2004), a larger predator is detected earlier by its prey and must swim at a higher absolute speed. However, the power required to overcome drag increases as absolute velocity increases (Somero and Childress, 1980). Accordingly, anaerobic metabolic potential (y), in pelagic fishes, scales with a positive coefficient (b) following a power relationship, $y=b_0M^b$, where b_0 is a normalization constant (Childress and Somero, 1990; Somero and Childress, 1980). The wide variation in coefficients for anaerobic capacity seen in fishes reflects the difference in predation strategies associated with divergent habitats, e.g. benthic versus pelagic (Childress and Somero, 1990).

Epipelagic squids, in particular of the families Ommastrephidae and Loliginidae, occupy oceanic and coastal ecological niches, respectively, similar to those of active predatory tunas and cods.

Like those fishes, squids visually seek out and capture fish and zooplankton as prey (Markaida and Sosa-Nishizaki, 2003; Nesis, 2002; Seibel et al., 2000). In fact, it has been postulated that the evolution of high performance in squids has resulted from competition, perhaps indirectly, with fishes (O'Dor and Webber, 1986; Packard, 1972; Seibel et al., 2000). Because of the similarity in ecological niche, we hypothesized that anaerobic metabolic capacity in pelagic squids should be high and, as in pelagic fishes, increase with body size.

Here, we examined anaerobic capacity in *Dosidicus gigas* (D'Orbigny 1835) (Family: Ommastrephidae) and *Doryteuthis pealeii* (Lesueur 1821) (Family: Loliginidae), two species of epipelagic squid. *Dosidicus gigas* is a relatively large squid found along the west coast of North and South America, with ranges extending out into the equatorial Pacific. They feed primarily on small myctophid fishes (Markaida and Sosa-Nishizaki, 2003) and follow a diel vertical migration pattern that mirrors that of their prey (Gilly et al., 2006). They spend extended periods of time within a layer of oxygen-depleted water at intermediate depths (~300 m), known as the oxygen minimum zone (OMZ) (Gilly et al., 2006). *Doryteuthis pealeii* (previously classified as *Loligo pealeii*) is a smaller Atlantic coastal squid. It reaches a smaller maximum size and does not undergo a diel migration like *D. gigas*, but does capture prey in a similar manner and therefore makes a useful comparative species. Both species are known to have high metabolic rates, surpassing those of predatorial fishes of similar size at comparable temperatures (Seibel, 2007).

RESULTS
Anaerobic metabolic potential was estimated from octopine dehydrogenase (ODH) activity measurements for *D. gigas* comprising six orders of magnitude of mass (0.16–17,200 g). The activity of ODH (y) ranged from 134 to 1253 U g^{-1} and decreased significantly ($P<0.0001$) with increasing size (M; $y=1768.2\times M^{-0.20}$; Fig. 1A, Table 1). However individuals <17.76 g had a slightly positive ($P<0.05$) scaling coefficient ($y=832.1\times M^{0.06\pm0.027}$; Fig. 1A, Table 1). The negative scaling observed in *D. gigas* is in stark contrast to the positive scaling seen in several pelagic species of fish (Fig. 1B). *Doryteuthis pealeii* ranging in size from 7 to 135 g showed a similar significant ($P<0.05$) negative scaling relationship ($y=172\times M^{-0.07\pm0.03}$). The highest activity for ODH of 1253 U g^{-1} in *D. gigas* is relatively low when compared with the LDH activity of ecologically similar pelagic vertebrates (Fig. 2). However, our results may be artificially low as a result of tissue sampling artifacts associated with the arrangement of red and white muscle equivalents in squids compared with fishes (see Materials and methods).

Theoretical scaling coefficients for power requirements of locomotion were estimated using Eqn 2 (see Discussion) and are presented in Table 2 along with measured scaling relationships for whole-organism activity of ODH for *D. gigas*. Assuming constant size-independent velocities (L^1, where L is length) across the size

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Fig. 2. Comparative glycolytic capacity. LDH activity of white tissue reported for several species of scombrid fish (Dickson, 1995) and ODH activity from whole-mantle cross-sections of *D. gigas* and *D. pealeii*. Maximum recorded activity rates were used for *D. gigas* and *D. pealeii*. The range of activities over varying body mass are presented in Table 1.

Fig. 1. Scaling of anaerobic activity. (A) Effect of body mass on octopine dehydrogenase (ODH) activity in *Dosidicus gigas* (filled squares) and *Doryteuthis pealeii* (open squares). (Regression equations are presented in Table 1.) (B) Comparative lactate dehydrogenase (LDH) activity for several species of epipelagic fish (Somero and Childress, 1980) compared with ODH activity in *D. gigas* and *D. pealeii*.

according to $L^{0.5}$. This demonstrates that *D. gigas* does not have sufficient ODH activity across its size range to support size-independent burst capacity, which suggests that smaller specimens require higher relative swimming velocities than larger ones.

The mean K_{pred} of *D. gigas*, the proportion of the body that is anterior to the deepest dorso-ventral cross-section of the body, is 0.608±0.007 (*N*=20). This is much higher than the mean values of 0.2–0.4 reported for teleost fishes and is equivalent to those reported for *Sphyraena barracuda* (Aleev, 1977; Domenici, 2002).

DISCUSSION
Anaerobic capacity
Our data show that *D. gigas* has some of the highest capacities for anaerobic metabolism amongst cephalopods, with ODH activities that range from 134 to 1252 U g^{-1} (Table 1). These values overlap with those of fellow ommastrephids *Symplectoteuthis oualaniensis* [1090 U g^{-1}; temperature corrected from 25°C using a Q_{10} of 2.0 (Baldwin, 1982)] and *Sthenoteuthis avalaniensis* [420.9 U g^{-1} (Seibel et al., 2000)]. However, they are much higher than those of

range, the calculated coefficients (*B*) are 4.0 for laminar and 4.6 for turbulent flow (Somero and Childress, 1980). In *D. gigas*, whole-animal ODH was proportional to $L^{2.8}$, which is consistent with power requirements only if swimming velocity decreases with size
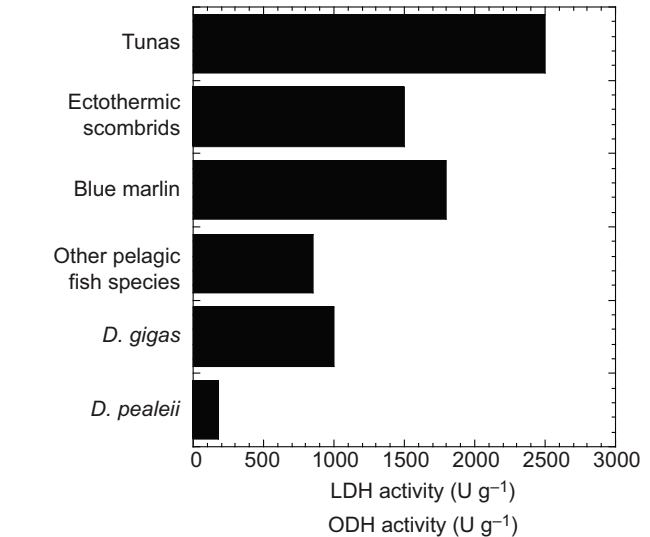
Table 1. Range of body size, enzymatic activity and power regression values for *Dosidicus gigas* (Ommastrephidae) and *Doryteuthis pealeii* (Loliginidae)

	Wet mass (g)	ODH activity (U g^{-1} wet mass)	ODH (=$b_0 M^b$) (µmol g^{-1} h^{-1})			
			b	b_0	r^2	*N*
Dosidicus gigas	17.8–17,200	134–1253	−0.20*	1768	0.83	30
	0.16–17.7		0.06**	832.1	0.16	27
Doryteuthis pealeii	7–135	90–159	−0.07	172	0.30	17
Gonatus onyx	1.20–31.08	29.82±7.26	−0.43	65.17	0.89	14
Abraliopsis falco	0.51–8.16	40.96±5.46				
Vampyroteuthis infernalis	0.45–1050	3.07±0.48	0.39	0.41	0.54	27
Sthenoteuthis oualaniensis	110	420.9				
Engraulis mordax	4.3–24.5		0.21	316.9		
Halichoeres bivittatus	2.1–39		−0.01	90.6		

Enzyme assays were performed at 20°C in substrate-saturating conditions. *Dosidicus gigas* with body mass >17.76 g; **D. gigas with body mass <17.76 g.
Enzymatic activity for comparative species was taken from previous publications (Seibel et al., 1998; Seibel et al., 2000; Rosa et al., 2009).
ODH, octopine dehydrogenase; b_0, normalization constant; *b*, coefficient; *M*, body mass.

Table 2. Scaling relationships of power to swim at velocities of L^1 and $L^{0.5}$ and enzyme activity compared with body length for *Dosidicus gigas* over 17.76 g

Relationship	b
Velocity scaled to $L^{0.5}$ in laminar flow	2.75
Velocity scaled to $L^{0.5}$ in turbulent flow	3.20
Velocity scaled to L^1 in laminar flow	4.0
Velocity scaled to L^1 in turbulent flow	4.6
Dosidicus gigas whole-animal ODH activity versus length	2.8

Scaling of power output was determined using Eqn 2.
L, length.

mesopelagic squids such as *Gonatus onyx* ($30\,\mathrm{U\,g^{-1}}$) and permanently deep-living species such as *Vampyroteuthis infernalis* ($3.1\,\mathrm{U\,g^{-1}}$) (Seibel et al., 2000). While the anaerobic activities we found for *D. gigas* are high among cephalopods, they are much lower than glycolytic enzyme activities reported for pelagic fishes. For example, tunas have LDH activities more than 2.5 times higher than the values reported for *D. gigas* here (Fig. 2) (Dickson, 1995).

Tissue composition must be considered when evaluating these enzymatic activities. In fish, most investigators compare LDH activities measured in isolated white muscle [see Somero and Childress for methods (Somero and Childress, 1980)]. By contrast, we sampled a core through the full thickness of the squid mantle muscle. Some species of squid have been shown to have thin layers of mitochondria-rich muscle tissue, analogous to vertebrate red muscle, toward the interior and exterior surfaces of the mantle (Mommsen et al., 1981). Mid-mantle tissue in squids thus may have higher activity of glycolytic enzymes than the interior and exterior mantle tissues (Mommsen et al., 1981). Values for ODH may effectively be diluted by inclusion of red muscle equivalents, causing a reduction in total ODH activity relative to white muscle tissue samples. However, the glycolytically poised core of the mantle muscle dominates the volume of the muscle at all adult sizes, at ~85% (Mommsen et al., 1981; Preuss et al., 1997).

The decrease in ODH activity per gram of total mantle muscle could be achieved either by reduced concentrations of glycolytic enzymes in mitochondria-poor (white) muscle or by a decrease in the relative mass of white muscle. The latter possibility suggests that there would be an increase in the relative mass of mitochondria-rich (red) muscle. Citrate synthase activity in *D. gigas* decreases with increasing size, but with a shallower slope (scaling coefficient=−0.19) than in fishes (Trueblood and Seibel, 2013), supporting the possibility of increased relative red muscle mass with size. Seibel (Seibel, 2007) reported an even shallower scaling slope for citrate synthase in active squids (b=−0.1), including *D. gigas* and other ommastrephids. As with ODH, the increased citrate synthase activity with size (relative to fishes) could be achieved via more or 'better' mitochondria or via increased red muscle mass (Dymowska et al., 2012). The latter possibility would displace white muscle tissue at larger sizes, which could play a role in the negative scaling coefficient for ODH in *D. gigas*. However, displacing white muscle is not without costs. For example, the Antarctic pteropod mollusk *Clione antarctica* elevates red muscle mass in locomotory muscles as compensation for cold temperature but at the expense of burst locomotory capacity (Dymowska et al., 2012; Rosenthal et al., 2009). Evidence suggests that the ratio of mitochondria-rich to mitochondria-poor muscle fibers does not change appreciably with size after an initial growth period in several species of squid (Moltschaniwskyj, 1994; Pecl and Moltschaniwskyj, 1997; Preuss et al., 1997). However, at least one species showed a decrease in the

ratio of mitochondria-rich to -poor muscle tissue with increasing size (Moltschaniwskyj, 2004). This suggests that the effect of whole-mantle sampling on the scaling relationships is probably small but requires further investigation.

Regardless, our measurements of the entire mantle core provide a measure of the total glycolytic capacity of the primary locomotory muscle. The reduced total anaerobic capacity at larger sizes is made possible by the use of tentacles for feeding and a reliance on relatively small prey at large size, which reduces the need for burst locomotory performance.

Epipelagic squids occupy the same niche as many of the top predatory fishes. Given the similarities, we hypothesized that squid should have similar power requirements and metabolic support to meet the demands of swimming at size-independent velocities (8–10 body lengths s⁻¹). However, we found that *D. gigas* and *D. pealeii* have lower anaerobic capacity, although this is partly a result of dilution with red muscle equivalents (see above), and display negative scaling coefficients (Fig. 1, Table 1). If squid were relying on size-independent swimming velocities to capture prey as fishes do, their whole-body anaerobic capacity should similarly increase with size. Using Eqn 1, Somero and Childress found that the power requirements for fish to travel at size-independent velocities (L^1) is proportional to L^4 for laminar, and $L^{4.6}$ for turbulent, flow. They found that whole-body glycolytic capacity as indicated by LDH activity scaled with body length at a value of 4, matching closely the increase in power output required for size-independent velocity. The whole-body ODH for *D. gigas* >17.76 g scales at $L^{2.8}$ (Table 2). This indicates that squid do not have the capacity to maintain size-independent burst swimming speeds, but instead that size-independent velocities decrease with size, proportional to ~$L^{0.5}$. We must therefore reject our original hypothesis and postulate possible explanations for diminished speed requirements in large squids (see below).

Within the class Cephalopoda, there are several examples of negative scaling of anaerobic capacity. The gonatid squid *Gonatus onyx* demonstrates an ontogenetic shift from positive to negative values of b (Hunt and Seibel, 2000; Rosa et al., 2009). This phenomenon has been attributed to ontogenetic changes in depth of occurrence. While juvenile *G. onyx* school and are negatively buoyant, active hunters in the upper 400 m of the water column, older, larger individuals are found in deeper water (700–800 m) and are neutrally buoyant, solitary hunters (Hunt and Seibel, 2000; Rosa et al., 2009). Low metabolic capacities in the deep ocean have been attributed to the low-light environment as outlined in the 'visual-interaction hypothesis' (Childress, 1995; Seibel and Drazen, 2007). The lack of light results in predator–prey interactions that take place over short distances and therefore need minimal burst energy (Childress, 1995; Seibel and Drazen, 2007; Seibel et al., 2000). Juvenile *G. onyx* that are in shallower water are visually exposed and require greater metabolic capacity to escape predators and capture prey. This may drive the positive b values in early life stages (Hunt and Seibel, 2000; Rosa and Seibel, 2008; Rosa and Seibel, 2010; Rosa et al., 2009). Negative scaling patterns have been shown in chiroteuthid and histioteuthid squids, as well bolitaenid octopods that ontogenetically migrate to greater depth (Seibel et al., 2000). However, the positive scaling coefficients reported for cranchid squids (Seibel et al., 1997) and vampyromorphs (Seibel et al., 1998) shows that, whatever the cause, it is not common to all cephalopods. Some potential causal traits include mode of transport, method of feeding (discussed below), or the mantle muscle arrangement discussed above.

Dosidicus gigas performs diel, but not ontogenetic, vertical migrations (Gilly et al., 2006). Life stages, from egg masses through

to large adults, have been found in shallow waters (<100 m) (Gilly et al., 2006; Staaf et al., 2008). Thus, we expected positive scaling throughout the size range of this species. In contrast, we found a breakpoint at 17.76 g and a clear decrease in anaerobic capacity at larger sizes (Fig. 1). This suggests that either there are several factors driving the scaling pattern of ODH in cephalopods as discussed below. These may include (1) tentacle use, (2) prey selection, (3) body shape and (4) rapid growth/large size.

Tentacle use

The use of tentacles to capture prey provides an advantage of decreased reliance on swimming as a squid increases in body size. Unlike fish, where success is limited by the ability to move the target (prey) into a gape-limited success area (the mouth) (Folkvord and Hunter, 1986; Juanes, 1994; Juanes and Conover, 1994), many cephalopods capture prey by extending long feeding tentacles, effectively increasing the area of strike success to the reach of the tentacles, which is much greater than gape. This allows less reliance on swimming as prey only have to be moved into the radius of the tentacle, not the mouth (Packard, 1972). By relying on tentacles, squid do not have to swim as far or as fast to capture prey. This likely plays a role in the comparatively low anaerobic capacity we have reported here (Fig. 2) (Dickson, 1995).

Increased body size also provides an advantage by decreasing reliance on swimming during prey capture. Prey selection is size independent in *D. gigas* (see 'Prey selection', below). As size increases, larger squid will have a longer reach with their tentacles relative to their prey, allowing prey to be captured at a greater distance. This results in larger individuals having to rely less on swimming and glycolytic demands to capture prey. This may contribute to the negative scaling observed in *D. gigas*' glycolytic capacity over 20 g body mass (~10 cm dorsal mantle length).

By increasing the distance between squid and prey, tentacle use also allows greater success in prey capture by decreasing stimulation of escape responses. Virtually all species of fish have a structure known as the Mauthner system that assists in the 'matador strategy' (Eaton and Emberley, 1991). The Mauthner system consists of fast-acting neurons that are mechano-acoustically, visually and chemo-tactically stimulated (Domenici, 2002). The mechano-acoustic segment is triggered by hair cells that are stimulated by waves coming off the leading edge of an approaching predator. This causes a rapid contraction of the muscles opposite to where the stimulus originated, moving the prey away from the predator (Abrahams, 2006; Canfield and Rose, 1996; Eaton and Emberley, 1991; Eaton et al., 1997). The visual component produces a similar escape response, stimulated by the rate of increasing size of the retinal image of an approaching predator as observed by the prey (Dill, 1974). A slowly approaching squid has a lower likelihood of triggering both the audio acoustic and visual components of the Mauthner system as it approaches. A large squid would be able to approach prey slowly and, at a comparatively greater distance, rapidly extend tentacles to grab prey. Adult *D. pealeii* have been shown to attack prey at the relatively slow velocity of ~4 body lengths s^{-1} (Kier and Leeuwen, 1997). During the final stage of prey capture, *D. pealeii* extends its tentacles toward prey at ~10 body lengths s^{-1} with remarkable accelerations of 250 m s^{-2}, ~100 body lengths s^{-2}. Our observations suggest that *D. gigas*' strike is similar to that of *D. pealeii*. Video observations show slow swimming approaches with rapid tentacle extension to capture prey. Tentacle strikes also allow prey strikes with diminished triggering of the Mauthner system by masking the approaching tentacle in the body cross-section behind it. This allows fast strikes by tentacles without increasing the reaction distance of the prey.

The use of tentacles to capture prey also allows squid to overcome angular momentum challenges. The matador strategy allows prey items to escape predators by remaining inside the radius of the smallest turn that the predator's momentum will allow. Because tentacles have a lower mass than the whole squid, they are more maneuverable and are able to turn in a smaller radius than the whole body traveling at similar speed. *Doryteuthis pealeii* has been documented to loop tentacles back around and grab prey when the initial strike does not contact the prey. We observed this in *D. gigas* (Fig. 3) (O'Dor, 2002). *Doryteuthis pealeii* are also able to curve the path of their tentacles during initial strike (Kier and Leeuwen, 1997). These two traits allow the tentacles to be maneuvered to follow the escape attempts of prey, and likely increase success rates of strikes.
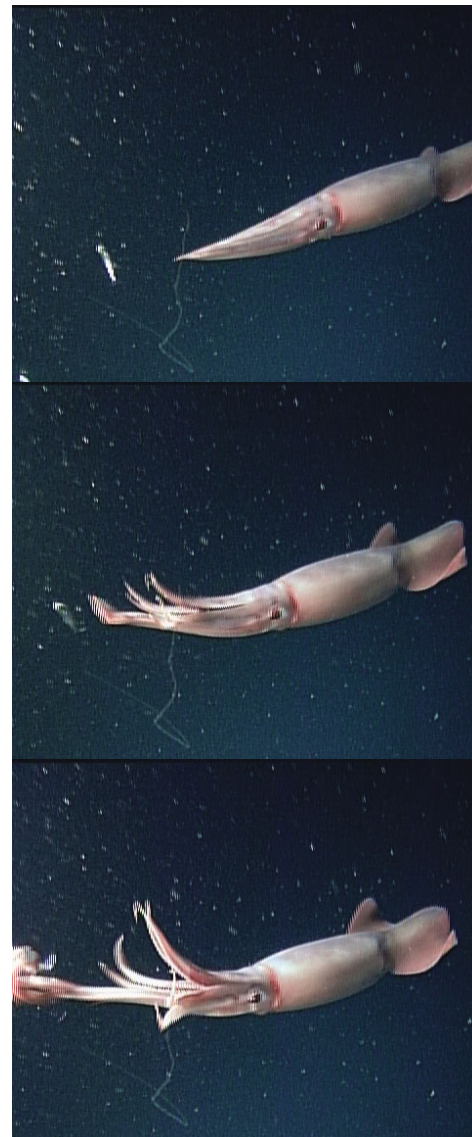

Fig. 3. Typical strike sequence of *D. gigas*. Footage was of an individual estimated at 5 kg, taken at ~300 m in the Monterey Basin, Monterey, CA, USA, using a remotely operated vehicle (DSRV Tiburon, Monterey Bay Aquarium Research Institute). Note, fins are extended during this sequence, a behavior that indicates relatively low swimming speeds (O'Dor, 2002). In the third frame you can see the club wrapping around to try to grab the prey fish as it escapes (see supplementary material Movie 1 for the complete sequence).

Prey selection

Prey selection is size independent in *D. gigas*. Unlike pelagic fishes that feed on increasingly larger prey as they grow, *D. gigas* feeds primarily on myctophid fishes ranging from 2 to 7 cm body length regardless of their size (Markaida and Sosa-Nishizaki, 2003). *Dosidicus gigas* does take non-fish items such as crustaceans, cephalopods and other zooplankton, but these tend to be small as well (Markaida and Sosa-Nishizaki, 2003). Because they select the same small-sized prey throughout ontogeny, the burst speed and power that *D. gigas* requires to match prey speed decreases exponentially with size. This decrease in speed and power demand allows for a decreased reliance on anaerobic energy sources during prey capture events. We hypothesize that this contributes to the negative glycolytic scaling seen in individuals larger than 17.76 g (Fig. 1A).

The decreasing disparity between predator and prey size may drive a change in prey selection that could explain the ontogenetic shift in ODH scaling at 17.76 g (Fig. 1A). For example, small *D. pealeii* show a preference for crustaceans, but as they increase in size there is a transition to piscivory, with prey that are generally larger than the crustaceans preyed upon at earlier life stages (Hunsicker and Essington, 2006). The negative scaling at larger size in *D. gigas* can be attributed in part to prey size. However, prey size is known to increase with squid body mass in *D. pealeii*, suggesting that other factors are at play (Moltschaniwskyj, 1994).

Body shape

Having a body shape that places the largest dorso-ventral body depth as far posterior to the mouth as possible provides an advantage during prey capture (Domenici, 2002). This diminishes closure time (T_C), the distance the predator has to swim to intercept the prey (DC), and the speed of the predator (U_{pred}). DC is equal to the reaction distance (RD) minus the proportion of the body length anterior to the largest body depth (K_{pred}) and predator body length (L_{pred}) (Domenici, 2002):

$$T_c = \frac{RD - K_{pred} L_{pred}}{U_{pred}}. \qquad (1)$$

The closer the mouth of a predator is relative to the deepest portion of the body, the earlier an escape response, relative to the mouth, is initiated (Domenici, 2002). For most teleost fish, K_{pred} ranges between 0.2 and $0.4L_{pred}$ and can reach as high as $0.6L_{pred}$ (Aleev, 1977; Domenici, 2002). Large K_{pred} values increase successful capture rates and allow predators to capture prey at slower velocities (Webb, 1982). Our measurements of *D. gigas* show K_{pred} at $0.608 \pm 0.007 L_{pred}$ (N=20) during strike postures. However, as tentacles extend past the arm tips during prey strike, K_{pred} increases and T_c decreases (Eqn 1), increasing the probability of successful capture (Fig. 4).

The cross-sectional shape of squid bodies provides them with an additional advantage. Elliptical and lenticular cross-sections trigger earlier escape responses compared with a rounded body cross-section (Webb, 1982). Piscivorous fish with rounded cross-sections have high capture rates (>80%) and low prey responses to attacks (28%), with apparent looming thresholds being 15–80 times larger (Webb, 1982). The high success rates are attributed to prey species responding less strongly to figures without apices. The cross-section of squid is circular, which should increase their relative capture success rate, unless the prey species has evolved to respond to the specific threat of predation by squids.

Predator avoidance

Dosidicus gigas are not only top predators but also important prey items for fishes (Smale, 1996) and marine mammals (Clarke, 1996).
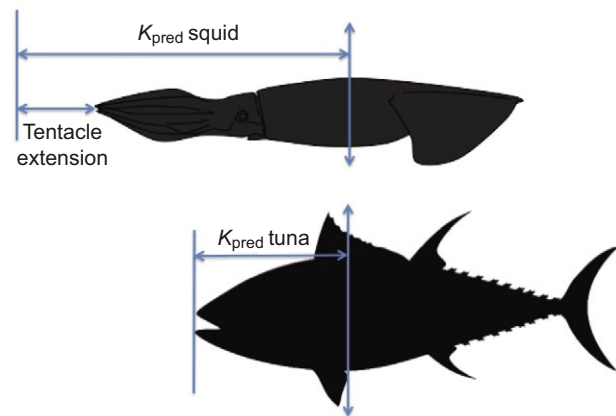

Fig. 4. Comparative K_{pred} of squid versus tuna. The closer the mouth of a predator is relative to the deepest portion of the body (K_{pred}), the earlier an escape response, relative to the mouth, is initiated (Domenici, 2002). Estimates of a 1 m squid and tuna show tuna would have a K_{pred} of 36 cm while the K_{pred} of squid would be more than twice that at 78 cm, assuming 1/3 mantle length as the tentacle extension length (see Kier and Leeuwen, 1997).

Pressure from these predators should drive anaerobic capacity sufficiently to avoid being preyed upon. However, there are several traits of both predators and prey that may diminish selective pressure on larger *D. gigas*.

Several fast-moving apex fishes regularly feed on *D. gigas* (Galvan-Magana et al., 2006; Smale, 1996). These fish tend to capture prey that are 10–20% of their body mass (Juanes, 1994; Scharf et al., 2000). However, there is a prey size prejudice in these fish, as they tend to choose smaller cephalopods than fishes because of handling issues (Staudinger et al., 2006). This bias for smaller cephalopod prey has also been demonstrated in sea birds (Croxall and Prince, 1996), seals (Klages, 1996) and smaller beaked whales (Clarke, 1996). Selection of smaller size by predators may lower selective pressure on larger *D. gigas* and play a role in the negative scaling of ODH in individuals over 20 g (Fig. 1).

Predation pressure is not completely removed from large squids as cetaceans and sharks commonly take large *D. gigas* as prey (Clarke, 1996; Smale, 1996). The predation pressure by fishes, such as sharks, is diminished in part by diel migratory patterns that place *D. gigas* in a dark, hypoxic layer of water known as the oxygen minimum zone (OMZ) that precludes lengthy forays by sharks and other top predators (Moltschaniwskyj, 2004; Pecl and Moltschaniwskyj, 1997). This refuge is incomplete as cetaceans are not affected by hypoxia and are able to track prey via sonar in the dark, and routinely enter and feed at the same depths as this barrier (Davis et al., 2007). The effect that these types of predator have on anaerobic capacity cannot be determined in this study; however, it is reasonable to assume that *D. gigas* maintains a level of burst capacity well above that of most marine animals, to escape attempted capture. The much higher activities of *D. gigas* compared with *D. pealeii* suggests that the open ocean requires greater burst swimming capacity for prey capture or predator avoidance than does the coastal environment occupied by *D. pealeii*. The coastal environment provides greater opportunities for crypsis and refuge and is generally a murkier optical environment that will diminish predator–prey detection distances.

Large size is achieved within a short lifespan by rapid growth in most cephalopods (Forsythe and Hanlon, 1988). *Dosidicus gigas*, especially, reaches sizes that preclude predation by most predators. Large size itself thus diminishes the need for burst escape swimming and may contribute to negative scaling.

Conclusions

Our data demonstrate that anaerobic metabolic capacity for *D. gigas* and *D. pealeii* scales differently from those reported for many active pelagic vertebrates. We suggest that method of prey capture, feeding behavior, prey selection and rapid growth primarily cause this divergence from the high anaerobic capacity and positive scaling patterns often observed in ecologically comparable fishes. Our data confirm the earlier findings of Childress and Somero (Childress and Somero, 1990) that behavior and ecology play an important role in setting anaerobic metabolic scaling.

Our data for *D. gigas* form one of the largest intraspecific studies performed, covering a size range of six orders of magnitude. This eliminates any challenges presented by phylogeny in interspecific studies (Symonds and Elgar, 2002). We suggest that there are many environmental, physiological and behavioral factors that influence metabolism, which cannot be ignored. Thus, metabolic rate and the mechanisms that cause scaling should be considered and treated as 'a many-splendored thing' (Suarez et al., 2004).

MATERIALS AND METHODS

Capture of animals

Animals were collected using four different methods. For *D. gigas*, individuals weighing more than 200 g were captured using hand lines and squid jigs. Animals weighing between 200 g and 1 g were captured using a hand-held dip net (area ~40 cm^2). Organisms smaller than 1 g were collected using an opening–closing Mother Tucker trawl with a 3 m^2 mouth fitted with a 30 l insulated cod end (Childress et al., 1978). For *D. pealeii*, animals larger than 40 g were collected using a hand line and squid jig. Those smaller than 40 g were caught using a casting net (1.5 m diameter). Collections of *D. gigas* took place in Guaymas Basin, Gulf of California, Mexico (27°N, 111°W) from the R/V *New Horizon* (Scripps Institute of Oceanography) in June of 2006–2007. *Doryteuthis pealeii* were collected from the Goat Island Bridge, Newport, RI, USA (41°N, 71°W) in April–May of 2006.

Animals larger than 500 g were weighed via a hand-held hanging scale to the nearest 0.01 kg. Smaller animals were weighed using a motion-compensated ship board balance (Childress and Mickel, 1980) while at sea, or frozen and weighed using a bench top balance upon return to the lab. After being weighed, tissue samples were taken from the anterior ventral mantle for enzyme analysis. The samples were immediately frozen in liquid nitrogen and maintained at −80°C until they were assayed.

Enzyme activity analyses

Mantle tissue samples were homogenized in varying dilutions of 0.01 mol l^{-1} Tris homogenization buffer, pH 7.5, at 10°C in Duall hand-held glass homogenizers kept on ice. Homogenate was centrifuged at 2500 **g** for 10 min at 4°C. Aliquots of 25 μl of sample supernatant were placed in 1.5 ml quartz cuvettes containing 1 ml Tris buffer under non-limiting substrate conditions. Maximal enzymatic activities (V_{max}) were measured with a Shimadzu UV-1700 spectrophotometer equipped with a water-jacketed cuvette holder connected to a recirculating water bath (Columbia, MD, USA). Measurements were carried out at 20°C and atmospheric pressure.

ODH (EC1.5.1.11) was assayed as an indicator of anaerobic metabolic capacity according to the methods of Baldwin and England (Baldwin and England, 1980). ODH catalyzes the terminal glycolytic reaction in cephalopods, resulting in the reductive condensation of pyruvate and arginine to form octopine. This reaction maintains redox balance in the cell when oxygen is limiting.

Drag analysis

By using a Newtonian drag model to estimate the power required to swim at a given velocity, it is possible to compare the power requirements for increased body length with the anaerobic capacity available to meet those power demands. Somero and Childress (Somero and Childress, 1980) used Webb's (Webb, 1977) drag model to demonstrate that anaerobic enzyme activity increased at the same rate as power output compared with body length in pelagic fishes:

$$P_t = D \cdot V = 1/2\rho \cdot S \cdot V^3 C_D, \qquad (2)$$

where P_t is the total power required to move the body through the water, D is total drag on the body, V is velocity with respect to the medium, ρ is the density of water, S is the wetted surface of the body (proportional to L^2) and C_D is the drag coefficient (Bainbridge, 1961). For laminar flow in the boundary layer:

$$C_D = \alpha 1.32 \cdot R_L^{-0.5}, \qquad (3)$$

and for turbulent flow over a smooth surface:

$$C_D = \alpha 0.078 \cdot R_L^{-0.2}, \qquad (4)$$

where R_L is the Reynolds number:

$$R_L = L \cdot V / v, \qquad (5)$$

where L is body length and v is the kinematic viscosity of water. Eqns 2–5 can be solved for relative velocity by substituting:

$$V = \text{constant} \cdot L^B. \qquad (6)$$

This same model was used on *D. gigas* to predict power requirements and compare with scaling of whole-body ODH activity.

K_{pred} calculations

Frame grabs from remotely operated vehicle video footage of *D. gigas* were printed and measured. Images were selected where individuals were perpendicular to the camera and arms were in a pointed cone posture. K_{pred} was determined by measuring the proportion of the body that was anterior to the deepest dorsal–ventral section of the body.

Statistical analysis

Power regressions of log-scaled enzyme activity and body mass were performed using JMP (SAS, USA) and significant results are reported as $P<0.05$. The ontogenetic break point in ODH activity of *D. gigas* was determined with a segmented analysis using R (Foundation for Statistical Computing, Austria) as described elsewhere (Muggeo, 2003). Means and standard errors of K_{pred} were determined using JMP.

Author contributions

L.A.T. participated in the conception, design, execution and interpretation of the data, and drafting and editing of the article. B.A.S. participated in the design, execution and interpretation of the data, and in revising the article.

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Movie 1. *Dosidicus gigas* feeding: this is a typical sequence of prey capture for *D. gigas* as observed from an ROV. Note in the last moments of the video that the club appears to curl back and grab the prey after initially missing the prey.