PHYSIOLOGY AND BEHAVIOR OF THE JUMBO SQUID DOSIDICUS GIGAS

BY

LLOYD A. TRUEBLOOD

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LLOYD A. TRUEBLOOD

APPROVED:

Thesis Committee:

Major Professor

DEAN OF THE GRADUATE SCHOOL

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ABSTRACT

Dosidicus gigas is a large, epipelagic squid that is found along North and South America and out into the equatorial pacific. Like other ommastrephid squids, *D. gigas* is known to vertically migrate, spending night hours on the surface feeding, and descending during day light into an area of oxygen deplete water known as the oxygen minimum layer (OML). This dissertation seeks to examine fundamental questions about physiological and behavioral adaptations to the environment in which *D. gigas* dwells.

Life in the open ocean is unique in that there is little to no refuge. As a result, prey are able to observe predators approaching. In order to capture prey, many pelagic fishes engage prey at high relative swimming speeds attempting to strike before prey detect them. This requires high metabolic rates and increasing anaerobic potential with increasing body size to over come resulting drag. Epipelagic squids are reported to have among the highest metabolic rates of any oceanic organism. I hypothesized that the activity of anaerobically poised enzymes would be high and increase with size as in ecologically similar fishes. In contrast, I demonstrate that anaerobic metabolic capacity in *D. gigas* is lower than high performance fishes and scales negatively with body mass. I attribute this to several cephalopod-specific traits, such as the use of tentacles to capture prey, and reduced relative prey size of adult squids. By decreasing reliance on burst swimming these traits may create a diminished reliance on anaerobically fueled activity during prey capture as predator size increases.

In order to determine strategies used to survive hypoxia in the OML, I developed a novel ship-board swim tunnel respirometer to measure aerobic metabolic

rates in conditions of the OML and surface waters. This swim tunnel respirometer allowed preliminary measurements of cost of transport in *D. gigas.* The broad size range of this species also enabled us to collect metabolic rates across 6 orders of magnitude. I found that *D. gigas* has one of the highest aerobic metabolic rates of any organism in the ocean, but there was a marked decrease of metabolism with decreased temperature. At environmental temperatures found in the OML, aerobic metabolism was higher than could be maintained given environmental oxygen content. The critical partial pressure (the oxygen partial pressure at which aerobic metabolism decreases) was similar to other cephalopods from the OML, but higher than the oxygen partial pressure in the OML. I observed that *D. gigas* was able to survive prolonged periods of anoxia (1-2 h) in a lethargic state. Our data suggests that *D. gigas* survives in the OML by suppressing metabolism. Preliminary cost of transport (COT) values were high in this species, which is likely the cause of the high routine aerobic metabolic rate I observed. This may also drive the shallow (less negative) scaling coefficients I observed, as squid do not appear to receive the same benefit of decreased locomotory costs with increasing size, as do fishes. Therefore *D. gigas* has proportionally larger aerobic metabolic capacity and associated aerobic metabolic rate with increased body size.

Cephalopods have the ability rapidly alter their external appearance through the use of chromatophores and body postures. Work describing these behaviors has focused on shallow, nearshore species dwelling in complex environments. This has led to the hypothesis that species from less complex environments will have a limited body pattern repertoire. Here I test this hypothesis by creating an ethogram for *D.*

gigas, a cephalopod from a simple environment. Observations via remotely operated vehicle (ROV) allowed us to record pattern components and their duration. I found this species has a catalog of components comparable in diversity, if not complexity, to cephalopods from the nearshore. Crypsis in the form of countershading occupied the majority of time observed. This suggests that crypsis for predator avoidance and prey capture is a selective factor for a range of body patterns in *D. gigas*. I discuss the functional roles the recorded body patterns have in the behavioral ecology of open ocean species.

This dissertation has provided answers to fundamental questions about the physiology and behavior of *D. gigas* in the open ocean and OML. I have demonstrated that physiological attributes result from behaviors that partially relieve the squids from energetic constraints as well as selective pressure from strong environmental gradients. Behavior in the form of body patterns was shown to be diverse yet suited for the visually simple environment in which these squid dwell.

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PREFACE

This dissertation is written in the manuscript format specified by the University of Rhode Island Graduate School and consists of three manuscripts.

Dosidicus gigas is a powerful, metabolically active epipelagic squid found along the west coast of the Americas, and out into the equatorial Pacific. They reach sizes in excess of 120 cm dorsal mantle length, and 50 kg mass. *Dosidicus gigas* plays a critical role as both prey and predator in the eastern tropical Pacific. Like other ommastrephids, *D. gigas* dwells in an open pelagic environment and performs diel vertical migrations, spending night hours in the surface feeding and descending to deeper water during daylight. In the course of this migration they encounter a broad temperature and oxygen gradient. The open pelagic realm, where this species is found, has little or no physical refuge and is considered to have low complexity. This study seeks to examine the physiology and behaviors of *D. gigas* in the open ocean and during its vertical migration.

Manuscript one presents the effect body mass (M) on anaerobic capacity (Y) according to the power equation $Y = b_0 M^b$ where b_0 is a normalization constant and b is the scaling coefficient. I explore the effect that tentacle use during prey capture and prey size selection has on this scaling relationship.

Manuscript two investigates the aerobic metabolic rate and cost of transport of *D. gigas*. I explored the effect of temperature and oxygen content on routine metabolic rate to determine how this species is able to survive daily exposure to hypoxia. I designed and fabricated a swim tunnel respirometer that was used to record the first estimates of cost of transport for this species. In the course of this study I was able to

obtain aerobic metabolic rates across 6 orders of magnitude of body mass. This allowed me to examine the effect of body mass on aerobic metabolic rates.

Manuscript three presents the behavior of *D. gigas* in the form of body patterns. I examine three components, color, posture, and locomotion, to determine the diversity of the repertoire of this open ocean species. The resulting ethogram is compared to those reported for nearshore species of cephalopods.

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CHAPTER 1

Slow swimming, fast-strike attacks: Effects of feeding behavior on scaling of anaerobic metabolism in epipelagic squid.

by

Lloyd A. Trueblood[1] ; Brad A. Seibel[2]

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[1]Ph.D. Candidate, Department of Biology, The University of Rhode Island, Kingston, Ri 02881. Email: Lloyd.trueblood@lasierra.edu

[2]Associate Professor, Department of Biology, The University of Rhode Island, Kingston, RI 02881. Email: Seibel@uri.edu

Abstract

Many pelagic fishes engage prey at high, size-independent, swimming speeds that require high metabolic rates and increasing anaerobic metabolic potential with increasing size to overcome the resulting drag. Epipelagic squids are reported to have among the highest metabolic rates in the oceans due to similarly demanding foraging strategies and the use of jet propulsion, which is inefficient relative to caudal fin undulation. This study examines enzymatic proxies of anaerobic metabolism in two species of pelagic squid, *Dosidicus gigas* and *Doryteuthis pealei* over six orders of magnitude size range. I hypothesized that activity of the anaerobically poised enzymes would be high and increase with size as in ecologically similar fishes. In contrast, I demonstrate that anaerobic metabolic capacity in these organisms is somewhat lower than high-performance fishes and scales negatively with body mass. I explore several cephalopod-specific traits, such as the use of tentacles to capture prey, adapted body morphology, and reduced relative prey size of adult squids, that may create a diminished reliance on anaerobically fueled burst activity during prey capture as size increases.

Introduction:

The shallow pelagic environment is unique in that there is little refuge from predation (Johnsen 2001; Robison 2004). As a result, visually cued predator-prey interactions take place over relatively long distances and visual predators maintain high metabolic and locomotory capacity as required by lengthy pursuit and evasion episodes (Childress 1995; Seibel, Thuesen et al. 2000; Abrahams 2006). Prey effectively use two tactics to avoid a predator once they have been detected in the pelagic environment (Abrahams 2006). The first, lesser used, is to let the predator know it has been detected and therefore, may not want to waste energy trying to attack (Sweatman 1984; Hasson 1991; Martin and Lopez 2001; Abrahams 2006). The second, most commonly used, strategy is to make an energetic decision to allow the predator to approach a certain distance before initiating an escape (Abrahams 2006). This strategy stops prey from prematurely making their presence known and may prevent a predator strike (Abrahams 2006). It also provides an advantage of maneuverability and angular momentum to the prey. As a larger predator approaches, the prey cannot out swim the predator over a long distance. Because the prey has lower mass it will have lower angular momentum. If it waits until the last possible moment and escapes at 90° from the angle of attack, the prey will stay inside the radius of the turn of the predator and escape capture (Abrahams 2006). This strategy, "like a matador avoiding a bull", (Hemingway 1932; Blaxter and Fuiman 1990) requires that pelagic predators build considerable speed with the hope of capturing prey before they initiate an escape response (Somero and Childress 1980; Childress and Somero 1990; Abrahams 2006).

Maximal speed is largely accepted as playing a key role in predator prey interactions (Hertz, Huey et al. 1988) . Pelagic fishes are characterized by high metabolic rates and size independent burst speeds during prey capture of ~10 body lengths sec^{-1} (Webb 1977; Wu 1977). These high relative speeds are presumably required across the size range to effectively capture prey. This is because the distance over which predators and prey detect each other increases with body mass (M). Thus, a larger predator detected earlier by its prey must swim at a higher absolute speed than a smaller predator. However, the power required to overcome drag increases as absolute velocities increase (Somero and Childress 1980). Accordingly, anaerobic metabolism (y), in some fishes, scales with a positive coefficient (*b*) following a power law, $y = b_0M^b$, where b_0 is a normalization constant (Somero and Childress 1980; Childress and Somero 1990). The wide variation in coefficients for anaerobic capacity reflects differences in predation strategies associated with divergent habitats e.g. benthic vs. pelagic (Childress and Somero 1990).

Many pelagic species of squid have a similar high-performance lifestyle. In particular, ommastrephid and loliginid squids have some of the highest aerobic metabolic rates among animals (Seibel, 2007; O'Dor and Webber, 1986). In fact, it has been postulated that the evolution of high performance in squids has resulted from competition, perhaps indirectly, with fishes (Packard 1972; O'Dor and Webber 1986; Seibel, Thuesen et al. 2000). Like pelagic fishes, squids visually seek out and capture fish and zooplankton as prey (Seibel, Thuesen et al. 2000; Nesis 2002; Markaida and Sosa-Nishizaki 2003). Because of the similarity in ecological niche, I hypothesize that

anaerobic metabolic capacity in pelagic squids should be high and, as in pelagic fishes, increase with body size.

Here I examine *Dosidicus gigas* (Family Ommastrephidae), and *Doryteuthis pealei* (Family Loliginidae) to determine if anaerobic capacity in pelagic squids scales as in pelagic fishes occupying a similar ecological niche. *Dosidicus gigas* is a large epipelagic squid with a very high metabolic rate and is an active predator (Seibel 2007). This species of squid is 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 their prey. They spend extended periods of time within a layer of oxygen deplete water at intermediate depths (~300m), known as the oxygen minimum layer (OML) (Gilly, Markaida et al. 2006). I collected specimens across 6 orders of magnitude in body mass, which is among the largest intra-specific size ranges used to date to investigate metabolic scaling. *Doryteuthis pealei* (previously referred to as *Loligo pealei*) is a smaller Atlantic coastal squid. It does not cover the same size range, or express a diel migration like *D. gigas*, but does capture prey in a similar manner and therefore makes a useful comparative species.

Methods:

Capture of animals

Animals were collected using four different methods. For *D. gigas,* individuals weighing more than 500 g were captured using hand lines and squid jigs. Animals weighing between 500 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, Barnes et al. 1978). For *D. pealei* 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 the Gulf of California, Mexico, *Doryteuthis pealei* were collected from the Goat Island Bridge, Newport RI, U.S.A.

Animals larger than 500 g were weighed via a hand-held hanging scale to the nearest 0.1 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 mantle of organisms that were used 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 *M* Tris homogenization buffer, pH 7.5 at 10°C in Duall hand held glass homogenizers kept on ice. Homogenate was centrifuged at 2500 X g for 10 min at 4°C. Aliquots of 25 µl of sample supernatant were placed in 1.5 ml quartz cuvettes containing 1ml 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 recalculating water bath. Measurements were carried out at 20 °C and atmospheric pressure.

Octopine dehydrogenase (ODH; EC1.5.1.11) was assayed as an indicator of anaerobic metabolic capacity according to the methods of 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.

Tissue composition must be considered when evaluating enzymatic activity of an organism. In fish, most investigators compare LDH activities measured in isolated white muscle (see Somero and Childress 1980 for methods). By contrast, I sampled a core through the full thickness of the squid mantle muscle. Some species of squid have been shown to have a thin layer of mitochondria-rich muscle tissue, analogous to vertebrate red muscle, toward the interior and exterior surfaces of the mantle (Mommsen, Ballantyne et al. 1981). This results in mid mantle tissue having a higher activity of glycolytic enzymes than the interior and exterior (Mommsen, Ballantyne et al. 1981). Our 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. The arrangement of muscle tissues in squids also creates the potential for the two enzymes to interact and influence each other. That ODH scaling varies throughout ontogeny and CS is consistent (b=-0.19 throughout ontogeny, Trueblood and Seibel in submission), suggests that the influence is negligible. Dilution of ODH values by

inclusion of red tissue may effect total ODH values, but should not affect the scaling coefficient, however further research is needed to verify this.

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 with increased body length to anaerobic capacity available to meet those power demands. In their 1980 publication, Somero and Childress used Webb's (1977) drag model to demonstrate that anaerobic enzyme activity increased at the same rate as power output compared to body length in pelagic fishes.

$$P_t = D \bullet V = \frac{1}{2} \rho \bullet S \bullet V^3 C_D \qquad (1)$$

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 in 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

$$C_D \, \alpha \, 1.32 \bullet R_L^{-0.5} \qquad (2)$$

and for turbulent flow over a smooth surface

$$C_D \, \alpha \, 0.078 \bullet R_L^{-0.2} \qquad (3)$$

where R_L is the Reynolds number:

$$R_L = L \bullet V/v \qquad (4)$$

where L is body length and v is kinematic viscosity of water. Equation (1-4) can be solved for relative velocity by substituting:

$$V = \text{constant} \bullet L^B \qquad (5)$$

This same model was used on *D. gigas* to predict power requirements and compare to 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 estimated by performing two power regressions at a visually estimated break point. The location of the break point was then moved by extending or reducing the largest mass range which was included in the early life-stage data-set (e.g. 0.16 g – X g). The resulting regressions that had the highest collective R^2 value were used to determine the actual break point. Means and standard errors of K_{pred} were determined using JMP (SAS, USA).

Results:

Anaerobic metabolic potential was estimated from ODH activity measurements for *Dosidicus gigas* comprising 6 orders of magnitude of mass (0.16 – 17200 g). The activity of ODH (y) ranged from 134-1253 units g^{-1} and decreased significantly ($p < 0.0001$) with increasing size (M) ($y = 1770 * M^{-0.20\pm0.03}$, Fig 1a, Table 1). However individuals <20 g had a slightly positive ($p < 0.05$) scaling coefficient ($y = 832.2 * M^{0.06\pm0.027}$, Fig 1a, Table 1). The negative scaling observed in *D. gigas* is in stark contrast to positive scaling seen in several pelagic species of fish (Fig 1b). Doryteuthis *pealei* ranging in size from 7-135 g showed a similar significant ($p < 0.05$) negative scaling relationship ($Y = 172 * M^{-0.07\pm0.03}$). The highest activity for ODH of 1253 units g^{-1} in *D. gigas* is relatively low when compared to ecologically similar pelagic vertebrates (Fig 2). However, our results may be artificially low due to tissue sampling artifacts associated with the arrangement of red and white muscle equivalents in squids compared to fish (see Methods section above).

Theoretical scaling coefficients for power requirements of locomotion were estimated using equation 1 and are presented in Table 2 along with measured scaling relationships for whole organism activity of octopine dehydrogenase for *Dosidicus gigas.* Assuming constant size independent velocities (L^1) across the size 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 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

I show that *D. gigas* has some of the highest capacities for anaerobic metabolism amongst cephalopods reported to date with ODH activities that range from 134 to 1252 units g^{-1} (Table 1). These values are higher than fellow ommastrephids *Symplectoteuthis oualaniensis* 1090 units g^{-1} (temperature corrected from 25°C using Q_{10} of 2.0) (Baldwin 1982) and *Sthenoteuthis avalaniensis* 420.9 units g^{-1} (Seibel, Thuesen et al. 2000). The contrast is even greater when compared to deeper occurring species such as *Gonatus onyx* 30 units g^{-1} and *Vampyroteuthis infernalis* 3.1 units g^{-1} (Seibel, Thuesen et al. 2000). While the anaerobic activities I report for *D. gigas* are high among cephalopods, they are considerably lower than rates reported for pelagic fishes. For example, tunas have LDH activates more than 2.5 times higher than the values reported for *D. gigas* here (Fig 2). Activities of aerobically-poised enzymes, such as citrate synthase, are similarly low relative to high-performance fishes (Trueblood et al., in prep; (Seibel 2007). While oxygen consumption rates measured in captivity are mostly higher than fishes, field metabolic rates indicate more energy-efficient behaviors in squids that reduce demand substantially (O'Dor and Webber 1986). This difference may be partly explained by the arrangement of red and white muscle equivalents in cephalopods, which hinders separate measurements of these muscle types as, discussed in methods.

Epipelagic squids occupy the same niche as many of the top predatory fishes discussed above. Given the similarities, I hypothesized that squid should have similar power requirements and metabolic support to meet the demands of swimming at size-

independent velocities (i.e. 8-10 body lengths sec^{-1}). However, I found that *D. gigas* and *D. pealei* display negative scaling coefficients (Fig. 1, Table 1). If squid were relying on size-independent swimming velocities as in fishes, their whole body anaerobic capacity should similarly increase with size. Using equation (1), Somero and Childress found that the power requirements required 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 lactate dehydrogenase activity (LDH) 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* > 20 g scales at $L^{2.8}$ (Table 2). This indicates that squid do not maintain size-independent burst capacity, but instead that velocities are proportional to $\sim L^{0.5}$, which shows a relative decrease in burst capacity with body size. I must therefore reject our original hypothesis.

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, Trueblood et al. 2009) This phenomena has been attributed to ontogenetic changes in depth of occurrence. While juvenile *G. onyx* school and are active hunters in the upper 400 m of the water column, older, larger individuals are found in deeper water (700-800 m) and are solitary hunters (Hunt and Seibel 2000; Rosa, Trueblood 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, Thuesen et al. 2000; Seibel and Drazen 2007). Juvenile *G. onyx* that are in shallower water are visually exposed and require greater metabolic capacity to escape predators and capture prey. This drives the positive *b* values in early life stages (Hunt and Seibel 2000; Rosa and Seibel 2008; Rosa, Trueblood et al. 2009; Rosa and Seibel 2010). Negative scaling patterns have been shown in chiroteuthid and histioteuthid squids, as well bolitaenid octopods that ontogenetically migrate to greater depth (Seibel, Thuesen et al. 2000). The occurrence of negative scaling of glycolytic enzymes in these groups of cephalopods suggests that it may be a cephalopod specific phenomenon. Some potential causal traits discussed below include mode of transport, method of feeding, or the mantle muscle arrangement discussed in methods and materials. However the positive scaling values seen in cranchid squids (Seibel, Thuesen et al. 1997) and in the order Vampyromorpha (Seibel, Thuesen et al. 1998) shows that, whatever the cause, it is not common to all cephalopods.

Dosidicus gigas performs diel, but not ontogenetic, vertical migrations (Gilly, Markaida et al. 2006). Life stages, from egg masses through large adults, have been found in shallow waters (<100 m) (Gilly, Markaida et al. 2006; Staaf, Camarillo-Coop et al. 2008). Thus I expected positive scaling throughout the size range of this species. My data do not support this, as there is a clear decrease in anaerobic capacity in individuals greater than 20 g mass (Fig 1). This suggests that either there are several factors driving the scaling pattern of ODH in cephalopods, or that light is not important. Below I discuss several squid/species specific phenomena, 1) prey

selection, 2) tentacle use, 3) body shape, and 4) rapid growth/large size that may explain the deviation from the expected results.

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 5-7 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 mollusks, but these tend to be small as well (Markaida and Sosa-Nishizaki 2003). Because they select the same sized prey throughout ontogeny, the burst speed and power *D. gigas* requires to match prey speed decreases exponentially with size (Fig 3). I hypothesize that this contributes to the negative glycolytic scaling seen in individuals larger than 20 g (Fig. 1a). The decreasing disparity between predator and prey size below 20 g may drive a change in prey selection that could explain the ontogenetic shift seen in our data (Fig. 1a). For example, small *Doryteuthis pealei* show a preference for crustaceans, but as they increase in size there is a transition to piscivory, with increasing prey size (Hunsicker and Essington 2006). The negative scaling at larger size in *D. gigas* can be attributed in part to prey size. However, the data for *D. pealei* would seem to suggest a broader phenomenon that does not depend solely on species-specific prey selection as prey size increases with body mass in this species. Feeding behavior and body morphology, as discussed below, are applicable to epipelagic squids more broadly and may further explain the observed scaling patterns.

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 target (prey) into a gape limited success area (mouth), (Folkvord and Hunter 1986; Juanes 1994; Juanes and Conover 1994) tentacle use effectively increases 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 moved into the radius of the tentacle, not the mouth (Packard 1972). Increased body size also provides an advantage in decreasing reliance on swimming during prey capture. As size increases, larger squid will have a longer reach with their tentacles, 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 contributes 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 may also allow 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" outlined above (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 being 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 (Eaton and

Emberley 1991; Canfield and Rose 1996; Eaton, Guzik et al. 1997; Abrahams 2006). 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 slow 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. pealei* have been shown to attack prey at the relatively slow velocity of ~ 4 body lengths sec^{-1}(CF: Kier and Leeuwin 1997). During the final stage of prey capture, *D. pealei* extends its tentacles toward prey at ~ 10 body lengths sec^{-1} with remarkable accelerations of 250 m s^{-2}, ~100 body lengths sec^{-2}. Our observations suggest that *D. gigas*' strike has similar patterns as *D. pealei* with video observations show slow swimming approaches with rapid tentacle extension to capture prey. Tentacle strikes also allow prey strike 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 fish.

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 able to turn in a smaller radius than the whole body traveling at similar speed. Thus, the tentacle can closely match the escape maneuvers of the prey. *Doryteuthis pealei* has been documented to loop tentacles back around and grab prey

when the initial strike does not contact the prey. They are also able to curve the path of their tentacles during initial strike if the trajectory is off (Kier and Leeuwen 1997). This is observable in *D. gigas* as well (Fig 4, personal observation). These two traits allow the tentacles to be maneuvered to follow the escape attempts of prey, and likely increase success rates of strikes.

Body shape

Having a body morphology with the largest dorso-ventral body depth as far posterior relative 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).

$$T_c = \frac{RD - k_{pred} L_{pred}}{U_{pred}} \qquad (6)$$

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 0.4 L_{pred} and can reach as high as 0.6 (Aleev 1977; Domenici 2002). Large K_{pred} values increase successful capture rates and allow predators with suboptimal body forms to capture prey at slower velocities (Webb 1982). Our measurements of *D. gigas* show K_{pred} at 0.608 (±0.007, N=20) during strike postures. However, as tentacles extend past the arm tips during prey strike K_{pred} increases. This increase diminishes T_c (equation 6) increasing the probability of successful capture.

The cross-sectional shape of squid bodies provides them with an additional advantage. Elliptical and lenticular cross-sections trigger earlier escape responses compared to 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 ALT's 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.

Predator avoidance

Jumbo squids are not only top predators, but are also important prey items for fishes (Smale 1996) and marine mammals (Clarke 1996). 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* (Smale 1996; Galvan-Magana, Olson et al. 2006). These fish tend to capture prey that are 10-20% of their body mass (Juanes 1994; Scharf, Juanes et al. 2000). However, there is a prey size prejudice in these fish, as they tend to choose smaller cephalopods than fishes due to handling issues (Staudinger, Juanes 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).

Predator 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 (discussed below) that place *D. gigas* in a dark, hypoxic layer of water known as the Oxygen Minimum Layer (OML) which sharks typically cannot enter. This refuge is incomplete as cetaceans are not effected 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, Jaquet et al. 2007). The effect these types of predator have on anaerobic capacity cannot be determined in this study, however, it is reasonable to assume that *D. gigas* must have some level of burst capacity to escape attempted capture. This would account for anaerobic capacity above that found in deep-sea cephalopods that do not experience similar predation.

OML

Dosidicus gigas spends daylight hours in an area of oxygen deplete water known as the oxygen minimum layer (OML) (Gilly, Markaida et al. 2006). This layer of water is marked by extremely low levels of oxygen (~0.1 ml liter^{-1}) which precludes most aerobically active organisms (Childress and Seibel 1998). And while seemingly sluggish at depth, *D. gigas* has been observed by remotely operated vehicle to forage and capture prey while in the OML (personal observation). The lights from the submersible may have an influence on this behavior, but the extent cannot be readily determined.

Organisms that dwell permanently in the OML are specialized for this environment and typically have low aerobic metabolic rates and physiological

adaptations that enhance O_2 uptake (Childress and Seibel 1998). It has been postulated that some cnidarians are able to survive periods in the OML without these adaptations, instead relying on anaerobic metabolism to meet metabolic demands (Thuesen, McCullough et al. 2005). However, the use of anaerobic metabolism to survive in the OML is associated with a positive scaling of glycolytic enzymes with body mass (Thuesen, McCullough et al. 2005). The exceptionally high-energy demand reported for *D. gigas* (Rosa and Seibel 2010) should outpace energy production via anaerobic pathways for prolonged time periods in the OML due to the relatively low anaerobic capacity of *D. gigas*. Observations of octopine production reveal a minor role of anaerobic metabolism and a strong reliance on metabolic suppression during forays into hypoxia (Rosa and Seibel 2010). Regardless of mechanism, the adaptations by *D. gigas* to survive in the OML may provide greater fitness by allowing this species to have lower exposure to predation. Tagging studies of swordfish, sailfish, and tuna have demonstrated that they typically do not enter the OML (Carey and Robinson 1981; Prince and Goodyear 2006). Most tuna, for example, are unable to tolerate oxygen levels below 2.0 ml liter^{-1} (Sharp 2001) which is well above the levels found in the OML in the Gulf of California (Trueblood and Seibel in prep). One species, *Thunnus obesus,* has anecdotally been shown to have a lower oxygen tolerance with blood P_{50} at or near oxygen partial pressures found in the OML (Lowe, Brill et al. 2000). The low oxygen levels found inside the OML may provide a physiological refuge that precludes large active predators with the exception of large marine mammals that are not affected by the OML. This, combined with

being in the low-light environment of the OML, may greatly decrease pressure from predation and allow for diminished burst capacity.

Conclusions

Our data demonstrate that squid anaerobic metabolic capacity scales differently than those reported for many active pelagic vertebrates. I suggest that prey selection, method of prey capture, feeding behavior, and body morphology causes divergence from the positive scaling patterns often observed in ecologically analogous fishes. Our data expand on and clarify the initial observations of Childress and Somero (1990) showing that behavior and ecology play an important role in setting anaerobic metabolic scaling.

Our data for *D. gigas* is one of the largest intraspecific studies performed, covering 6 orders of magnitude size range. This eliminates any challenges presented by phylogeny in interspecific studies (Symonds and Elgar 2002). I 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, Darveau et al. 2004).

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Table 1: Range of body size, enzymatic activity and power regression values for *Dosidicus gigas* (Ommastrephidae) and *Doryteuthis pealei* (Loliginidae). Enzyme assays were performed at 20°C in substrate-saturating conditions. Data with * indicate *D. gigas* with body mass > 20 g, ** indicates *D. gigas* with body mass <20 g. Enzymatic activity for comparative species were taken from Seibel et al., (1998); Seibel et al., (2000); and Rosa et al., (2009).

Table 1

	Wet mass (g)	Octopine dyhdrogenase activity (units g^{-1} wet mass)	ODH = b_0M^b (μmol g^{-1} h^{-1}))			
			b	b_0	r^2	N
Dosidicus gigas	20-17200	134-1253	-	1770	0.81	28
	0.16-20		0.20*	832.2	0.16	29
			0.06**			
Doryteuthis pealei	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				

Table 2: Scaling relationships of power to swim at velocities L^1 and $L^{0.5}$ and enzyme activity compared to body length for *Dosidicus gigas* over 20 g. Scaling of power output was determined using equation 1.

Table 2

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
D. gigas whole ODH Vs. length	2.8

Figure 1: A) Effect of body mass on ODH activity (units g^{-1}) *Dosidicus gigas* (closed squares) *Doryteuthis pealei* (open squares). Regression equations are presented in Table 1. B. Lactate dehydrogenase (units g^{-1}) for several species of epipelagic fish (Somero and Childress 1980) compared to the ODH in *D. gigas* and *D. pealei*.

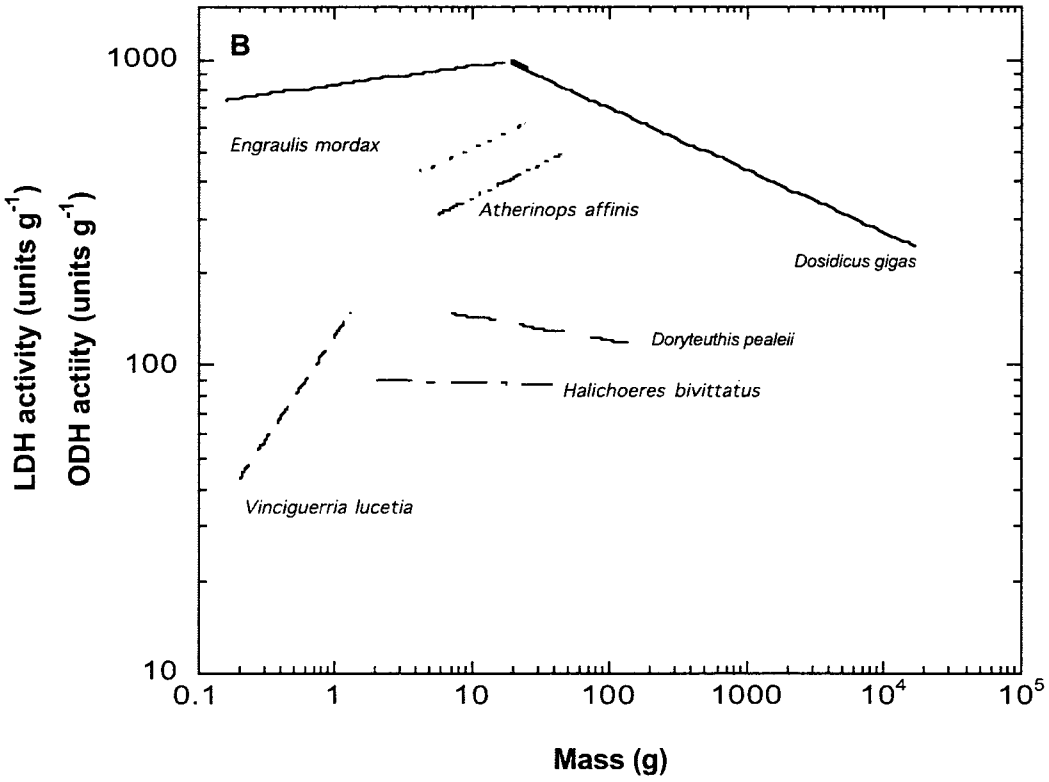
B
1000
100
10
0.1
1
10
100
1000
10^4
10^5
LDH activity (units g^{-1})
ODH actiity (units g^{-1})
Mass (g)
Engraulis mordax
Atherinops affinis
Dosidicus gigas
Doryteuthis pealeii
Halichoeres bivittatus
Vinciguerria lucetia

Figure 1

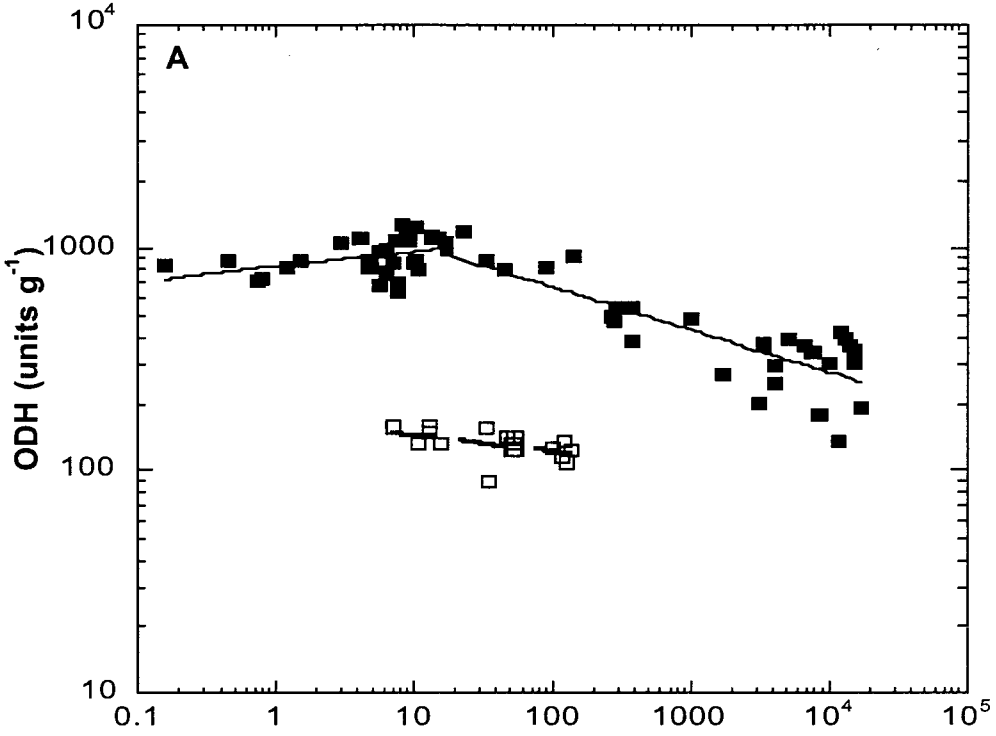

Figure 2: LDH activity (units g^{-1}) of white tissue reported for several species of scombrid fish (Dickson 1995) and ODH activity (units g^{-1}) from whole mantle cross sections of *D. gigas* and *D. pealei*. Maximum recorded activity rates were used for *D. gigas* and *D. pealei*. Range of activities over varying body mass are presented in Table 1.

Figure 2:

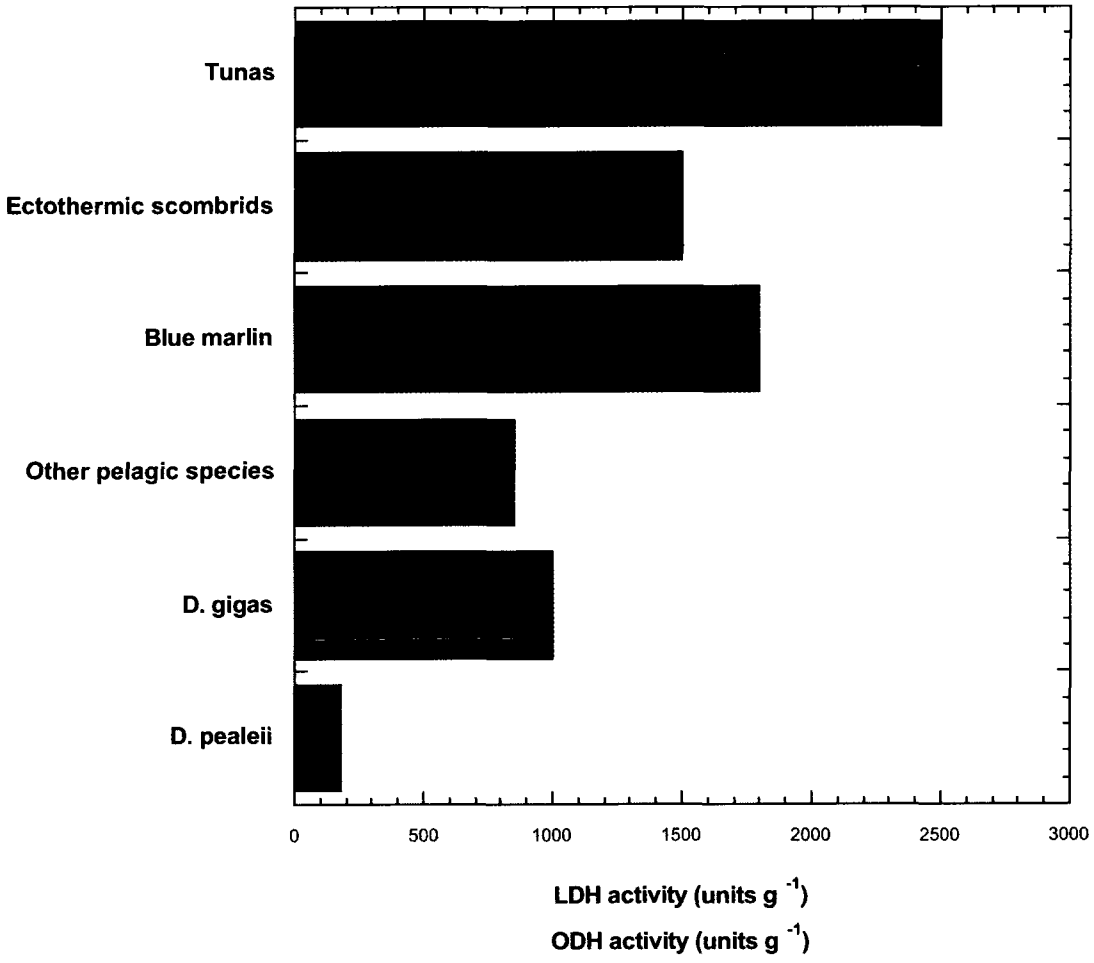
Tunas
Ectothermic scombrids
Blue marlin
Other pelagic species
D. gigas
D. pealeii
0
500
1000
1500
2000
2500
3000
LDH activity (units g -1)
ODH activity (units g -1)

Figure 3: Relative velocity in terms of body lengths sec^{-1} for *D. gigas* to match the speed of a 5 cm prey item swimming at the burst speed of 10-body lengths sec^{-1}. The exponential decrease in relative velocity demonstrates *D. gigas* is able to swim at low relative velocities and still match prey species absolute velocity.

Figure 3:

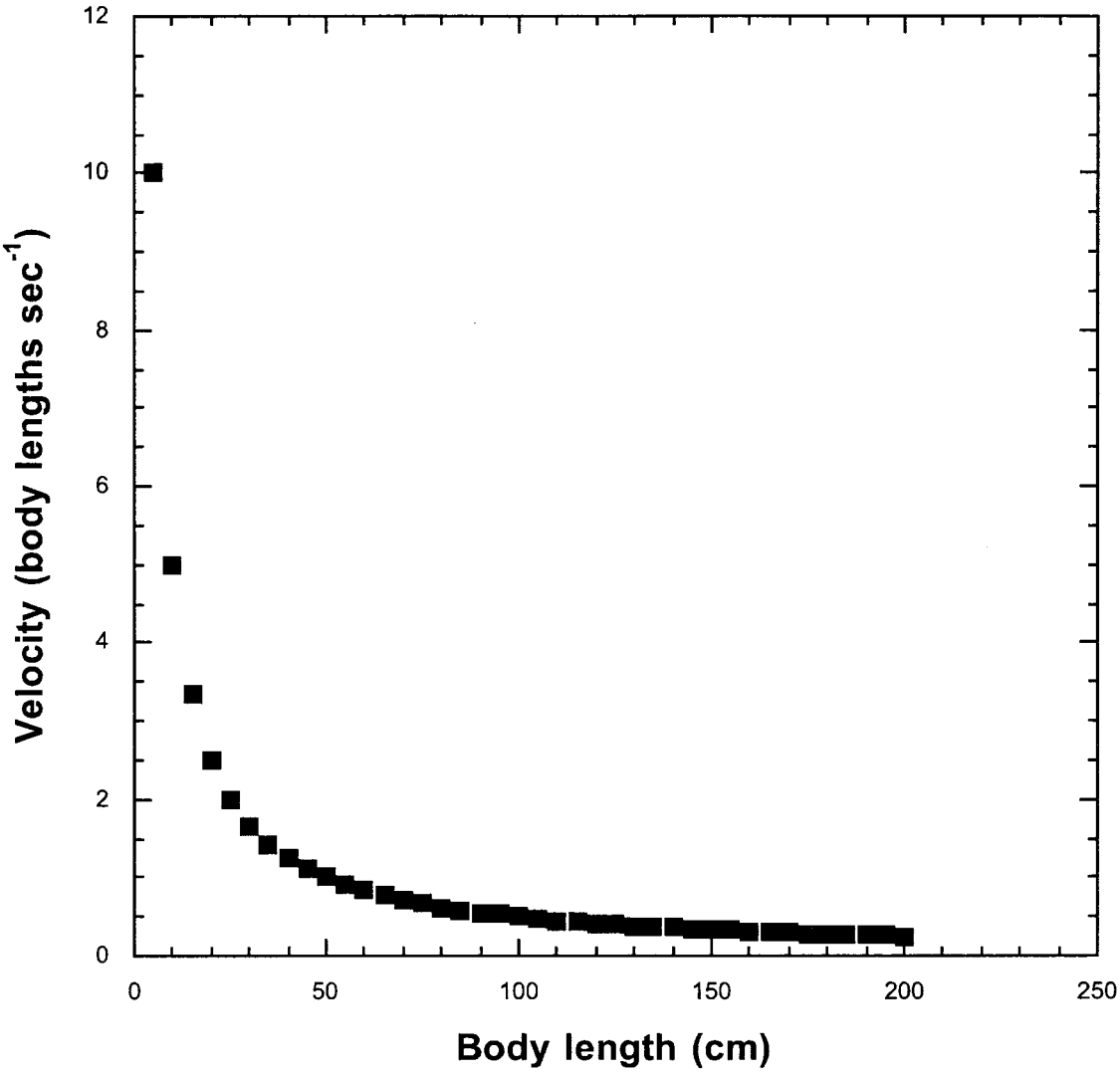
Velocity (body lengths sec^{-1})
Body length (cm)
0
2
4
6
8
10
12
0
50
100
150
200
250

Figure 4: Frame grabs from video footage of *Dosidicus gigas* taken from a remotely operated vehicle (DSRV Tiburon, Monterey Bay Aquarium Research Institute). Footage was of an individual estimated at 5 kg, taken at ~300 m in the Monterey Basin, Monterey California, U.S.A. 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.

Figure 4

Chapter 2

Oxygen Consumption of the Vertically Migrating Jumbo Squid *Dosidicus gigas* (Ommastrephidae) Recorded in a Portable Ship-board Swim Tunnel Respirometer

by

Lloyd A. Trueblood[1] ; Brad A. Seibel[2]

In prep for submission to the Journal of Experimental Biology

[1]Ph.D. Candidate, Department of Biology, The University of Rhode Island, Kingston, Ri 02881. Email: Lloyd.trueblood@lasierra.edu

[2]Associate Professor, Department of Biology, The University of Rhode Island, Kingston, RI 02881. Email: Seibel@uri.edu

Abstract

Dosidicus gigas is a large, metabolically active, epipelagic squid found in the eastern Pacific. This species is known to vertically migrate into a pronounced oxygen minimum layer (OML) that is expected to be physiologically prohibitive. Here I introduce a novel ship-board swim tunnel respirometer to measure metabolic rates of adults at OML and surface conditions, as well as the cost of transport (COT). The broad size range of this species allowed us to collect metabolic rates across 6 orders of magnitude body mass. *Dosidicus gigas* (2 -17 kg) expressed a mean routine metabolic rate (RMR) of 5.54 μmol g^{-1} h^{-1} at 10°C and 11.68 μmol g^{-1} h^{-1} at 20°C. At 20°C *D. gigas* had a critical partial pressure, the oxygen partial pressure where aerobic metabolic rate can no longer be maintained (P_{crit}) of 3.8 kPa, and at 10°C P_{crit} = 1.6 kPa. I recorded COTs of 60 and 18 J kg^{-1} m^{-1} on a 10 and 11.9 kg squid respectively. Oxygen consumption rate (Y) varied with body mass (M) according to $Y = 11.18\ M^{-0.11\pm0.03}$. Citrate synthase activity varied with body mass according to $Y = 9.32M^{-0.19\pm0.02}$. These values were found to be significantly different from proposed universal scaling values. Metabolic rates and P_{crit} are above levels that can be supported with the amount of environmental oxygen present in the OML. Thus, metabolic suppression may be a means for survival while in the OML. The preliminary high cost of transport in large individuals likely drives the shallow (less negative) scaling coefficients I observed, as active muscular squid do not appear to receive the same benefit of decreased locomotory costs with increasing size, as do fishes. *Dosidicus gigas* must have a proportionally larger metabolic capacity, and associated metabolic rate, with increased body size.

Introduction

Dosidicus gigas is a large, metabolically active epipelagic squid found along the west coast of the Americas, and out into the equatorial Pacific (Nesis 1983; Seibel 2007). They reach sizes in excess of 120 cm dorsal mantle length, and 50 kg (Nesis 1983). *Dosidicus gigas* plays a critical role as both prey (Clarke and Paliza 2001; Davis, Jaquet et al. 2007) and predator (Markaida and Sosa-Nishizaki 2003) in the eastern tropical Pacific, and it is estimated that they remove 4 million tonnes of food (primarily myctophid fish) per year assuming daily intake of 3% adult body mass (Shulman, Chesalin et al. 2002; Markaida and Sosa-Nishizaki 2003).

Like other ommastrephids, *D. gigas* performs diel vertical migrations, spending night hours at the surface feeding and descending to deeper water during daylight (Gilly, Markaida et al. 2006). During this migration they encounter a broad temperature and oxygen gradient (Gilly, Markaida et al. 2006; Rosa and Seibel 2008). The oxygen concentration reaches a minimum (< 5 μmol O_2 l^{-1}), termed the oxygen minimum layer (OML), at intermediate depths of approximately 300 to 800 m. The OML is strongest in areas of high productivity, such as the eastern tropical Pacific, and can extend hundreds of meters in depth and span thousands of kilometers in area (Kamykowski and Zentara 1990). Permanent residents of the OML, including many cephalopod species, typically have physiological adaptations, such as large gill surface areas, enhanced ventilatory abilities, and respiratory proteins with high oxygen affinity, that enable them to maintain aerobic metabolism in this hypoxic environment (Childress and Seibel 1998). Although not a specific adaptation to hypoxia, most

permanent residents are also marked by low aerobic metabolic rates (Childress 1995; Seibel, Thuesen et al. 1997; Childress and Seibel 1998; Seibel and Drazen 2007).

Ommastrephids have exceptionally high metabolic rates (Seibel 2007) associated with their inefficient mode of jet propulsion (O'Dor and Webber 1986). Such high rates are paradoxical, considering the limitation in their oxygen delivery system. Haemocyanin, the respiratory pigment used by cephalopods, is a large extracellular molecule that is found in low concentrations compared to the hemoglobin of fish, due to viscosity constraints (O'Dor and Webber 1986; Portner 2002). Thus, oxygen-carrying capacity is low in squid compared to active fish. Squids consume virtually all arterial oxygen from the blood on each pass through the body (Portner 2002). Additionally, haemocyanin in active squids studied to date has a low oxygen affinity to promote the release of oxygen at the tissues (Bridges 1994). These oxygen transport properties of active squid are thought to place them chronically on the edge of oxygen limitation, even in well oxygenated water (O'Dor and Webber 1986; Finke, Portner et al. 1996; Portner 2002). An estimated 60% of total oxygen is taken up cutaneously, which in concert with optimized circulatory performance (Shadwick, O'Dor et al. 1990; Portner 2002), allows squid to be as metabolically active as high-performance heterothermic fishes (Seibel and Drazen 2007).

High metabolic rates, and constrained oxygen delivery, should preclude *D. gigas* from entering the OML during its daily migration. However, preliminary work by Gilly et al. (2006) showed hypoxia tolerance and recovery in three individual *D. gigas* (weighing between 1.1 and 7.2 kg) at 6-7°C. Each squid repeatedly consumed available oxygen from a closed respiration chamber to levels below 10 $\mu mol\ l^{-1}$ and

was able to recover with no apparent distress. Metabolic rate decreased once oxygen partial pressure reached a critically low level (P_{crit}) at 1.5 kPa. They postulated that the resulting decrease in oxygen uptake was indicative of metabolic suppression. However, while aerobic metabolic rates did show a decrease, the energy required could be made up anaerobically and may not represent a true metabolic suppression where total ATP usage is diminished.

Rosa and Seibel (2010) found a significant decrease in oxygen consumption from 20°C (17.8 μmol O_2 g^{-1} h^{-1}) to 10°C (8.9 μmol O_2 g^{-1} h^{-1}) in juveniles (1-50g). When exposed to hypoxia the oxygen consumption rate at 10°C dropped further from 8.9 μmolO_2 g^{-1} h^{-1} to 1.6 μmolO_2 g^{-1} h^{-1}. Examination of anaerobic end products resulting from hypoxia exposure showed *D. gigas* makes up only a small fraction (~7.0%) of its energy deficit expected in the OML anaerobically. Therefore, a substantial suppression of total ATP production (between 64% and 87% at 10°C) must occur under hypoxic conditions in the OML. The vertical and horizontal migrations, and the accompanying gradients in environmental conditions, of adult *D. gigas* are likely more pronounced than those juveniles. Furthermore, body mass is known affect both aerobic and anaerobic metabolic capacity. *Dosidicus gigas* body mass spans over 6 orders of magnitude, providing a distinct advantage of examining the effect of body mass on metabolism without the confounding challenges presented by phylogeny (Symonds and Elgar 2002). The physiology of adult *D. gigas* has not been addressed in detail.

Here, I introduce a novel ship-board swim tunnel respirometer (Fig. 1) that I used to measure the metabolic rates of adults up 10 kg at the temperature and oxygen

levels consistent with day and night depths. I also provide preliminary data on the effects of swim speed on jet pressure and metabolic rate that I used to estimate the cost of transport in large squids. I examine the relationship of body mass (M) and metabolic rate (Y) according to the power equation $Y= b_0M^b$, where b_0 is a normalization constant and b is a scaling coefficient which describes the slope. The results are discussed in the context of causal mechanisms that may drive the scaling coefficients observed in epipelagic squids and provide insight into the vertical physiology of these important predators.

Methods

Daytime distribution

Footage from the Monterey Bay Aquarium Research Institute's (MBARI) video collection was examined for occurrences of *D. gigas*. This library contains 15,000 + hours of video footage and was created using the ROVs *Ventana* and *Tiburon*. Video footage from the ROVs was examined for the number of *D. gigas* seen within a given depth range. Depths were binned into 100 m increments. The frequency of occurrence was determined by dividing the number of *D. gigas* seen by the amount of time the ROV spent in each depth bin. Footage was divided between observations made in Monterey Basin and observations made in the Gulf of California

Capture of animals

Animals were collected using four different methods. Individuals weighing more than 500 g were captured using hand lines and squid jigs. Animals weighing between 500 g and 1 g were captured using a hand held dip net (area ~40 cm^2). Organisms smaller than 1 g were collected using either opening-closing Mother Tucker trawl with a 3-m^2 mouth fitted with a 30 L insulated cod end (Childress, Barnes et al. 1978), or by hand via blue water scuba diving.

Collections of squid took place in the Gulf of California, Mexico, in the Guaymus basin, 27° 15.01' N, 111° 30.38' W, off of Santa Rosalia 27° 16.74' N, 112° 8.22' W, and in Carmen Basin 26° 14.39' N, 110° 42.24' W. Tissue samples were collected also from the Monterey Basin, California 36° 33' N, 123° 24' W. Squid larger than 1 kg were only collected the Monterey Basin.

Animals larger than 500 g were weighed via a hand held hanging scale to the nearest 0.1 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.

Mantle length and thickness were measured at the time of capture. Dorsal mantle length was measured using a tape measure to the nearest 0.1 cm. Mantle thickness was measured with digital calipers (Fisher Scientific) to the nearest 0.1 mm.

After individuals were weighed and measured, tissue samples were taken from the mantle for enzyme analysis. The samples were immediately frozen in liquid nitrogen and maintained at -80°C until they were assayed.

Organisms used for both oxygen consumption and enzymatic measurements were first placed in their respective respirometery chambers immediately after capture and allowed to acclimate (see below). After respiration experiments were performed, organisms were weighed and tissue samples were taken.

Oxygen consumption

Oxygen consumption rates were measured using three different techniques . Organisms smaller than 1 g were place in 50 ml glass syringes or 300 ml BOD bottles used as a closed respirometery chamber (Marsh and Manahan 1999). Animals were incubated in 0.2 μm filtered seawater with 50 mg/l streptomycin to control microbial growth. Control chambers were kept without animals. Syringes were incubated in a recirculating, refrigerated, water bath to control temperature. The syringes were kept covered to avoid stimulation of animal behavior by light. Oxygen content was measured by injecting seawater samples past a Clarke-type oxygen electrode in a

water-jacketed housing. Respiration rate was determined by subtracting oxygen content measurements in animal chambers from the controls (Marsh and Manahan 1999).

For organism weighing between 1 and 50 g, we used a 200 ml flow through respirometer was used. Water was circulated through the chamber via a peristaltic pump. A water-jacketed reservoir was bubbled continuously with air to maintain incoming water at high PO_2 and desired temperature. Oxygen content of the water exiting the chamber was subtracted from the oxygen content of the water entering the chamber. The resulting oxygen consumed was divided by body mass and duration (hrs) and multiplied by volume of flow rate to determine $\mu mols\ g^{-1}\ hr^{-1}$. The resultant data is published in Rosa and Seibel (2010).

Organisms weighing more than 500 g were placed in either a 513 L water-jacketed closed respirometer or water-jacketed swim tunnel respirometer. The closed respirometer consisted of a sealed aquarium placed in a temperature regulated water-jacket (an 800 l fiberglass tub). Water temperature was maintained via an external chiller (Delta Star, Model DS-9). Water was pumped through tygon tubing into the chiller and returned to water-jacket via a high flow water pump (IWaki America, Model MD-1100 RLT 115). Oxygen content was measured directly via a Clarke-type oxygen electrode.

The swim tunnel respirometer (Fig. 1) consisted of a rectangular working section (1.7 m X 0.55 m X 0.40 m) made from an "off the shelf" waste holding tank. The inlet to the working section had 7.6 cm thick section of grids with 1 cm squares to help smooth out flow into the working section. At the rear of the working section

there was a 20.6 cm diameter PVC pipe connected to a 30 cm bow thruster. Additionally there was a video camera (Sea View, model SMM-50-SYS) at the rear of the working section to allow observations of squid while swimming.

The bow thruster was powered by a 2 hp DC motor (Dayton DC, model 1F804). The thruster created water flow at the desired velocity (0-0.30 m sec^{-1}). The tunnel was submerged in a large temperature regulated fiberglass water bath (2000 l, foot print = 3.15 m X 1.32 m). Temperature was maintained via an external chiller (Delta Star, Model DS-10). Water was pump through tygon tubing into to the chiller, and returned to the water bath via a high flow water pump (IWaki America, Model MD-1100 RLT 115). Water was pumped via a peristaltic pump through tygon tubing from the inlet and outlet of the swim tunnel to an external oxygen electrode (Strathkelvin Instruments) and then returned to the respirometer allowing for continuous measurement of oxygen consumption.

Oxygen content at the outlet was subtracted from oxygen content at the inlet to determine the oxygen consumed per liter. The resulting value was multiplied by the flow rate (l sec^{-1}) to determine oxygen consumed at a given water velocity. This was then used to calculate cost of transport (J m^{-1} s^{-1}). An external temperature regulated reservoir was maintained with oxygen saturated seawater. When the oxygen levels of the swim tunnel became too low, the water from the reservoir was pumped into the tunnel, and water from the tunnel was drained off until oxygen level reached the desired value.

This flow-tunnel was designed specifically to fit inside the aft wet lab of the R/V New horizon for data collection at sea. The tunnel and water bath are attached to

two aluminum I-beams allowing them to be easily crated and transported anywhere in the world. Only one other flow tunnel has been used at sea (Graham, Dewar et al. 1990) it is considerably larger (3.3 m X 5.3 m) despite a similar sized working section. It cannot be used inside the shape and must be placed on the back deck at the mercy of the elements. Our entire apparatus was built from off the shelf materials, costing ~$15,000 and was constructed by The Equipment Development Lab at the University of Rhode Island.

Routine metabolic rates

The rates presented here are termed routine metabolic rate (RMR) because spontaneous activity in the closed respiratory chambers could not be controlled in most cases but all rates were measured under conditions that minimized activity to the greatest extent possible. Experiments were carried out at 10 °C or 20 °C, individuals were only tested at one temperature. Animals were allowed to acclimate for several hours and kept in darkened chambers.

Cost of transport

Large individuals (~10 kg) were placed in the swim tunnel respirometer described above. They were observed via low light video camera placed in the rear of the swim tunnel (Fig 1). The DC motor was turned to the desired velocity (0.10-0.25 m sec^{-1}), and squid were observed swimming. For calculations of the cost of transport, oxygen consumption rates were taken only during periods when the squid were clearly swimming without contacting the bottom of the swim tunnel.

Enzyme activity analyses

Mantle tissue samples were homogenized in varying dilutions of 0.01 *M* Tris homogenization buffer (pH 7.5 at 10°C) in Duall hand held glass homogenizers kept at 4°C on ice. The homogenate was centrifuged at 2500 X Gravity for 10 min at 4°C. Aliquots of 25 µl of the sample supernatant were placed in 1.5 ml quartz cuvettes containing 1ml 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 recalculating water bath. Measurements were carried out at 20 °C and atmospheric pressure for consistency with previous studies.

Citrate synthase, an important regulatory enzyme in the first step of the Krebs cycle (Hochachka, Storey et al. 1975) (CS; EC.4.1.3.7) was used as a proxy for aerobic metabolism. It was assayed according the methods of Thuesen and Childress (1994).

Because of the inclusion of enzymatic data that are inherently mass specific (measured by grinding a sample of muscle tissue rather than the entire organism) I have chosen to show oxygen consumption rates and enzymatic activity in terms of mass specific metabolism (µmols g^{-1} h^{-1} or units g^{-1} h^{-1} respectively). Whole-animal metabolic rates lead to the same conclusion and can be calculated by multiplying rates by the mass values listed in Table 1.

Pressure tags

Pressure tags were attached inside the mantle by inserting two needles through the mantle, being held in place by two tightly fitting rubber washers (0.8 cm). Pressure

was measured differentially using a semiconductor strain gauge bridge mounted in a stainless-steel housing filled with light mineral oil. Pressure was recorded using methods outlined in Webber and O'Dor (1986).

Statistical data analyses

A Students t-test was used to determine if there was a significant difference in the mean depth of occurrence between the Gulf of California and the Monterey Basin. Power regressions were performed on oxygen consumption vs. body mass and citrate synthase activity vs. body mass using Kaliedagraph (Synergy Software, USA). Analysis of covariance (ANCOVA) was performed on JMP (SAS Institute, USA) on effects at 10°C (n = 6) and 20°C (n = 5) on RMR with body mass as a covariant. The resulting means were used to establish a Q_{10}, (the effect a 10°C increase in temperature has on RMR). An ANCOVA was also performed on citrate synthase activities from Gulf of California and Monterey Basin data with body mass as a covariate. Significant results are reported at the 95% confidence level.

Two methods were used to calculate P_{crit}. First I plotted RMR vs. P_{O2} for experiments at 10°C (n = 6) and 20°C (n = 5) and determined the P_{crit} for each run. These were combined and the mean and standard error were then calculated for each temperature on JMP (SAS Institute, USA). This method showed that at 20°C P_{crit} = 3.4 kPa ± 0.27 and at 10°C P_{crit} = 1.9 kPa ± 0.51. Additionally, I binned RMR at 0.1 kPa intervals throughout the range of P_{O2} for both temperatures. The mean and SE of RMR from each bin was determined. The resultant means were then plotted, and P_{crit} was determined. Using this method at 20°C, *D. gigas* had a P_{crit} = 3.8 kPa, and at 10°C P_{crit} = 1.6 kPa. Because of variation in the data as some experimental runs were

stopped early, and some experiments did not give a clear P_{crit} I have chosen to use the results from the second method. The data falls within one SE calculated using the first method, and so can be considered similar.

Results

Remotely operated vehicle video footage confirmed that *D. gigas* frequents depths consistent with the OML during daylight hours in the Monterey Basin and the Gulf of California (Fig. 2). *Dosidicus gigas* was found deeper in the Monterey Basin (mean = 537 m) compared to Gulf of California (mean = 440 m) ($p < 0.0001$).

Dosidicus gigas weighing between 2 and 17 kg had a mean RMR of 5.54 (±1.31) μmol g^{-1} h^{-1} at 10°C (n=6) and 11.68 (±1.44) μmol g^{-1} h^{-1} at 20°C (n = 5). These values were shown to be significantly different (ANCOVA, $F_{1,5} = 5.36$, $p < 0.05$). An example of a typical respiration run (mass = 7 kg) can be seen in Figure 3. The squid was able to survive complete anoxia for almost 2 hours. Death was considered when coordinated mantle contractions ceased. Survival for longer than an hour in complete anoxia was common in most of the large individuals I examined.

By plotting oxygen consumption rate at the environmental oxygen partial pressure we found that at 20°C *D. gigas* had a P_{crit} = 3.8 kPa, and at 10°C P_{crit} = 1.6 kPa (Fig. 4) for 2-17 kg individuals. Partial pressure of 3.8 kPa occurred ~ 125 m depth, and 1. 6 kPa, at ~250 m (Fig. 4).

Oxygen consumption was used as a proxy for COT assuming 20 J mlO_2^{-1}. I completed two consumption measurements during active locomotion that were verified via the video system (60 and 18 J kg^{-1} m^{-1} on a 10 and 11.9 kg squid respectively).

Mass specific oxygen consumption rates of D. *gigas* were measured for individuals across 6 orders of magnitude of body mass (Table 1, Fig. 5A). The oxygen consumption rate (Y) varied with body mass (M) according to $Y = 11.18\ M^{-}$

$^{0.11\pm0.03}$. The mean + 1 standard error (SE) of the slope (-0.11 ±0.03) did not include the commonly reported scaling coefficients –0.25 or –0.33. Citrate synthase activity varied with body mass according to $Y = 9.32M^{-0.19\pm0.02}$ (Table 1, Fig. 5B), and mean ± 1 SE of the slope (-0.19 ± 0.03) did not include –0.25 or –0.33. Enzymatic activity and oxygen consumption were not measured from the same specimens.

I plotted allometry measurements of mantle length and thickness against body mass on a log-log scale to determine if *D. gigas* follows surface areas to volume ratio reported for other cephalopods. Power regressions yielded length = $3.31M^{0.33}$, r squared = 0.91 and thickness = $0.90M^{0.34}$ r squared = 0.78, (ANCOVA, $F_{1, 219} = 488$, $p < 0.0001$). The similarity of the slopes shows that *D. gigas* does not benefit from having a greater surface area to volume ratio with increased size.

Discussion

Temperature/Critical partial pressure

The metabolic rates reported here for *Dosidicus gigas* are among the highest recorded for any oceanic organism (Seibel and Drazen 2007). Such rates have been reported for several species of epipelagic squid (reviewed in Seibel, 2007). Such high values have led some to postulate that active squid, such as *D. gigas,* should be limited to oceanic regions with high oxygen concentrations and moderate temperatures (Portner 2002). However, our observations of *D. gigas* in the Monterey basin via ROV (Fig. 2), and tagging studies in the Gulf of California (Gilly, Markaida et al. 2006), show *D. gigas* present at depths where oxygen concentration are uniformly low in the pronounced oxygen minimum zone.

At 20°C in normoxic water, I found adult *D. gigas* consume 11.68 μmol O_2 g^{-1} h^{-1}. At this pace, a 1 kg squid in the OML (~ 0.23 kPa, Fig. 4B) would have to pump 5056 L of sea water per hour, extracting 100% of the oxygen from seawater. This is a virtual impossibility given the physical coupling between ventilation and jet propulsion in cephalopods (Wells 1990). Such a high ventilation rate can only be achieved at high swimming speeds that further drive up oxygen demands. While D. gigas' RMR is reduced in the OML by a factor of about 2 (Q_{10} = 2.1) due to decreased temperature at depth, the reduction does not fully alleviate the constraints imposed by the extreme hypoxia in the OML. At 10°C, a 1 kg *D. gigas* would still have to pump 1971 L of seawater, assuming complete extraction of all available oxygen. Thus, suppression of RMR beyond that provided by temperature is required if metabolic demand is to be met aerobically in the OML.

Dosidicus gigas is able to maintain a constant RMR throughout an oxygen gradient until a critically low oxygen partial pressure (P_{crit}) is reached, at which point RMR decreases. I found a P_{crit} of 3.8 kPa at 20° C and 1.6 kPa at 10°C (Fig. 4A). This is consistent with the initial findings of Gilly et al., (2006 P_{crit} of 1.5, n = 3, 6-7°C) . Animals from environments with stable oxygen concentrations tend to have P_{crit} values matched to the lowest oxygen partial pressures experienced in their environments (Childress and Seibel 1998). *Dosidicus gigas*' P_{crit} value is higher compared to other cephalopods found in less well developed OMLs, such as that found off California (Fig. 6). Its P_{crit} is also higher than the lowest oxygen partial pressure experienced in the Gulf of California (Fig. 4B). Our CTD data show that the water at 25 m depth is 20°C and has an oxygen partial pressure of 19.9 kPa (Fig. 4B). This does not present a physiological challenge. However, oxygen partial pressure is 0.2 kPa at 370 m, the depth where 10°C is reached (Fig. 4B). Tagging studies of Gilly et al., (2006) in the Gulf of California show *D. gigas* spends the majority of daylight hours between 200-300, temperature ranges between 11.2 and 12.7°C with oxygen partial pressure ranging 0.33 and 1.03 kPa. This is well below the P_{crit} I found at 10°C. Therefore, *D. gigas* is spending a considerable amount of time in an environment where its RMR cannot be maintained aerobically.

The ability of juveniles to tolerate oxygen concentrations below P_{crit} has been attributed to metabolic suppression rather than to an increase in anaerobic ATP production (Rosa and Seibel 2008; Rosa and Seibel 2010). Curtailing activity levels and costly cellular processes presumably achieve this state of hypometabolism. For example, reduction of protein synthesis can reduce cellular energy demand by almost

30% (Guppy and Withers 1999; Hochachka and Somero 2002). Cessation of ion transport also greatly reduces the demand for ATP in hypoxic cells (Hochachka and Somero 2002).

I observed that *D. gigas* was able to tolerate prolonged exposure to anoxia at both 10°C and 20°C. Individuals were able to completely deplete oxygen from the water in the respirometers and survive up to two hours of total anoxia (Fig. 3). Movement was greatly diminished and the squids were lethargic, lying on the bottom of the tank. *Dosidicus gigas* became lethargic during extreme hypoxia and anoxia. This behavior supports the hypothesis that metabolism is suppressed in the OML. However, survival over such a long period suggests that there may be anaerobic pathways producing ATP. Rosa and Seibel (2010) determined that anaerobic metabolism, as indicated by the accumulation of octopine, did occur but was well below values need to meet energy demands. Squid may use alternative anaerobic pathways for ATP production during prolonged hypoxia. For example, anaerobic protein catabolism has been hypothesized in ommastrephid squid based on low O:N ratios in hypoxic conditions (Shulman, Chesalin et al. 2002). Anaerobic pathways which rely on amino acids have also been described in several bivalve mollusks (De Zwaan 1983; Hochachka and Somero 2002). Additionally, *D. gigas* could be producing volatile compounds such as ethanol, which can then be passed across the gill (Hochachka and Somero 2002). Further exploration is needed to determine which, if any, of these anaerobic pathways are being used by *D. gigas* during hypoxia exposure.

Preliminary COT

The environment in which an organism dwells has a considerable impact on its metabolic rate. For example, because light diminishes with depth in the ocean, deep-living marine organisms that rely on vision to capture prey tend to interact with predators and prey over short distances. As a result, these organisms have lower metabolic rates than their shallow living relatives (Childress 1995; Seibel and Drazen 2007). The lack of refuge in the well-lit epipelagic zone requires comparatively high energetic demands, because predator-prey interactions take place over long distances and require energetically expensive high speed swimming (Childress 1995; Seibel and Drazen 2007). Accordingly, top epipelagic predators are under strong selection for high metabolic capacity and muscle performance to achieve high speeds required (Carey, Teal et al. 1971).

Epipelagic squid have been shown to have exceptionally high metabolic rates, amongst the highest of any ectotherm (Seibel 2007; Rosa, Trueblood et al. 2009). Ostensibly this makes sense, as epipelagic squid are visual predators foraging in the pelagic environment, seemingly relying on speed to capture prey like the aforementioned top predators. However, the reliance on high speed burst swimming to capture prey by the epipelagic squid *Dosidicus gigas* has recently been questioned by Trueblood et al (2009). They propose that tentacle use and selection of small prey allow for low whole body velocities during prey capture. This may drive the low, and negatively scaled, glycolytic capacity for this species compared to other top pelagic vertebrate counterparts. If these squid are not relying on rapid swimming to capture

prey like other top pelagic predators, they should have comparatively low aerobic metabolic rates.

The high metabolic rates reported for epipelagic squid are linked to their use of jetting for locomotion (Wells 1990). Limited data suggests that the cost of transport (Y) scales with body mass (M) at $Y=aM^{-3}$ in fish and $Y = aM^{-2}$ in squid (O'Dor and Webber 1986). Thus at small size, jetting is more efficient than fin swimming, but as size increases, COT in squids surpasses that of fishes (O'Dor and Webber 1986). My first two estimates of COT for large *D. gigas* vary (18 and 60 J kg^{-1} m^{-1} swimming at 0.25 m sec^{-1} for individuals near 10 kg body mass; Fig. 7) but seem to confirm previous estimates of the high cost of transport and the shallow scaling slope for those costs. Preliminary data from pressure tags placed inside the mantle cavity show high jet pressures similar to those reported for *I. illecebrosus*, lending further support to the hypothesized energetic expense of jet propulsion (Fig. 8).

Scaling of Aerobic Metabolism

Mass-specific metabolic rates (y) of organisms typically decline with increasing body mass (M) according to $y = aM^b$, where a is a normalization constant and *b* is a scaling coefficient that describes the slope of the relationship. Epipelagic squid families were found to have greater (less negative) scaling coefficients than many organisms, including deeper living and benthic cephalopods (Seibel 2007). Thus, body mass had only a modest effect on mass-specific metabolism in epipelagic squids. Our study extends the size range of *D. gigas* for which metabolic rate measurements are available to 6 orders of magnitude, removing the phylogenetic challenges posed by interspecific comparisons (Symonds and Elgar 2002). I found

that RMR and CS activity had scaling coefficients (b) of -0.11 and -0.19 respectively. Confidence limits for both data sets did not include -0.25, sometimes reported as a universal scaling coefficient (West, Brown et al. 1997). Several feasible but non-exclusive mechanisms have been presented that could explain the shallow scaling values observed (Seibel 2007).

For example, the divergent scaling relationships for COT in fishes and squids (discussed above). Alternatively, unique surface area to body mass relationships in some species of epipelagic squid may influence scaling patterns. Epipelagic squids have unique body geometry of a hollow cone, where surface area does not decrease, relative to body mass, with increasing size as in most other solid bodies (O'Dor and Hoar 2000). As they increase in size, the inner and outer surfaces areas increase. However, mantle thickness does not increase as fast as length, thus surface area increases relative to mass as size increases (O'Dor and Hoar 2000). Because some cephalopods have been shown to respire cutaneously through mantle surfaces (Madan and Wells 1996; Portner 2002), the increase in effective gas exchange surfaces with size would alleviate potential oxygen uptake constraints (Seibel 2007). While cutaneous respiration is no doubt important in *D. gigas*, I show that the mantle thickness increases with mass at the same rate as mantle length suggesting that the SA:V consideration cannot explain the unusual scaling relationships I observed.

Conclusion

In conclusion, I have shown that *D. gigas* has an exceptionally high RMR that should preclude it from the pronounced OML in the Gulf of California. Furthermore, critical oxygen partial pressures were well above minimum environmental oxygen

partial pressure experienced during daytime excursions to depth. Available evidence suggests squid are able to survive such excursions via metabolic suppression, but some contribution of anaerobic ATP synthesis is also apparent. Further exploration needs to be done in this area to fully understand the role of anaerobiosis in surviving hypoxia in this species. The preliminary high cost of transport I recorded likely drives the shallow (less negative) scaling coefficients I observed, as active muscular squid do not appear to receive the same benefit of decreased locomotory costs with increasing size as do fishes. Therefore, *D. gigas* must have a proportionally larger metabolic capacity and associated metabolic rate with increased body size.

Critique of methods

The swim tunnel design outlined above does an excellent job of providing a swimming platform that can readily be brought aboard most medium and large UNOLS vessels. It is easily transportable, able to be crated and shipped around the world to meet ships at ports where science teams are being picked up. However, there are some limitations resulting from the compact nature of the design.

Of primary concern is the consistency of flow through the working section. The present design does not provide laminar flow in the working section. Because water coming out of the thruster is turning at the end of the water bath to enter the working section, the flow on the outside has a greater velocity than that on the inside, causing gradation of water velocity laterally across the working section. I observed that swimming squid tended to swim on the inward (more central) section, which is

the area of lowest flow velocity. The swim tunnel is presently being presently modified to address these issues.

Large squid were readily able to deplete the oxygen in the swim tunnel, even when an air-stone was placed in the swim tunnel between experimental runs. An external water tank (760 l) was used as temperature regulated reservoir for oxygen-saturated water that could be pumped into the swim tunnel to replace de-oxygenated water. If the tunnel is being used on organisms, such as squid, which can release ink into the water, an even greater volume is required to clear the fouled water when the squid release ink. If ship constraints do not allow such a large volume of water on deck or in the wet lab, a bank of several serial linked chillers could be used to cool ambient sea water as it is pumped aboard directly to the swim tunnel.

I suspect that the variation in flow rate resulted in large errors in the 60 J kg^{-1} m^{-1} data point. The lower rate of 18 J kg^{-1} m^{-1} is high, but much closer to the 10.4 J kg^{-1} m^{-1} reported for *Illex illecebrosus* a fellow ommastrephid, and several other cephalopods swimming at similar velocities (Fig. 7A).

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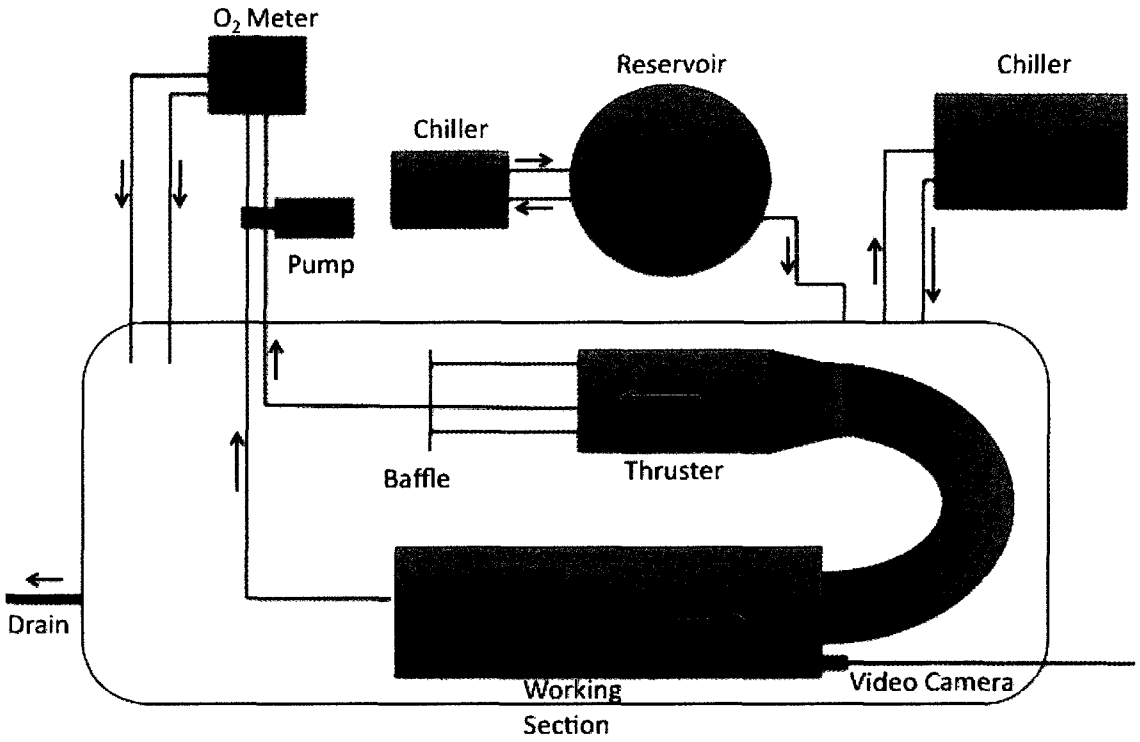
Table 1: Range and scaling of oxygen consumption and citrate synthase activity in *Dosidicus gigas*.

Table 1

	Wet mass (g)	O_2 (μmol g^{-1} h^{-1}) CS (units g^{-1} wet mass)	$Y = b_0M^b$			
Oxygen Consumption	0.0095-10000	2.24 – 27.64	b -0.10	b_0 11.18	r^2 0.43	N 90
Citrate Synthase	0.16-17200	0.98 – 11.4	-0.19	2.39	0.84	58

Figure 1:
A) The temperature regulated swim tunnel used to measure cost of transport (top view). A video camera (red) enabled observations of squid while in the working section (B). See text for details. NEED TO LABEL A, B on figures

Figure 1:


O_2 Meter
Reservoir
Chiller
Chiller
Pump
Baffle
Thruster
Drain
Working
Section
Video Camera

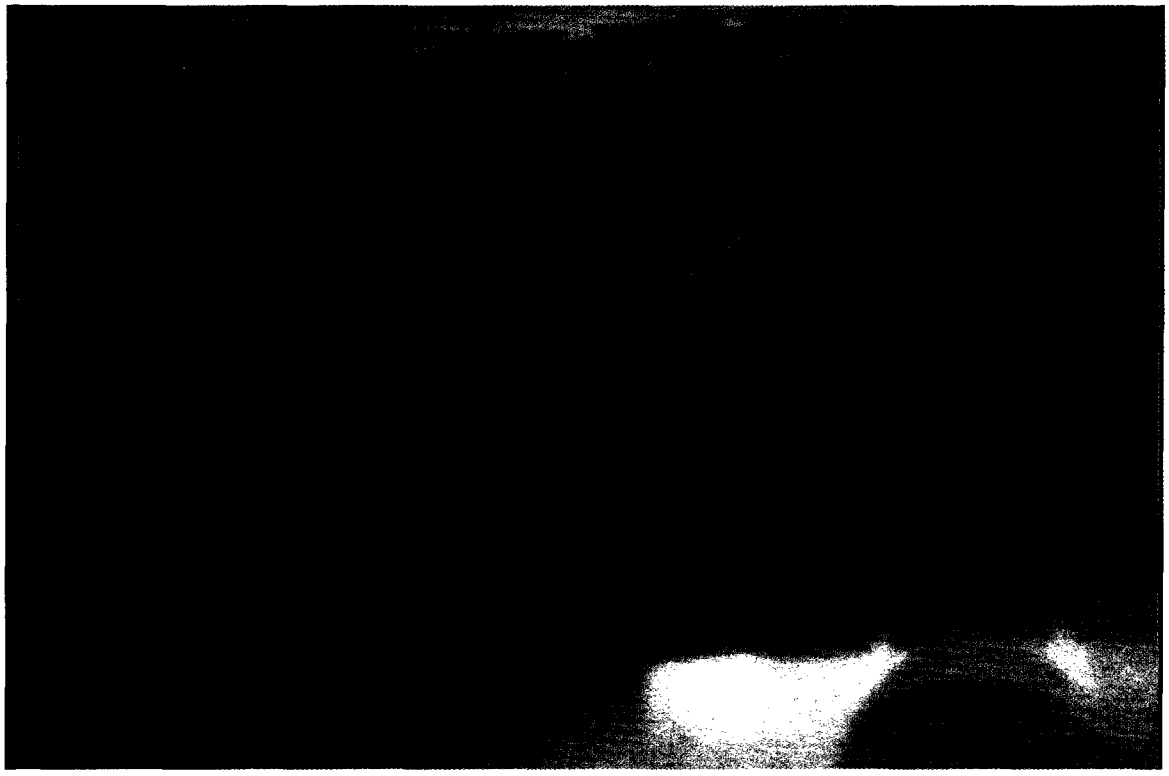


Figure 2:
Vidoe footage of *Dosidicus* gigas from the Monterey Bay Aquarium Research Institute was used to determine the depth of occurance during dives. The depth a sat which a squid was observed was recorded. This data was binned at 100 m intervals. The number of squid observed at each bin depth was divided by the time (h) the ROV spent at each depth to determing number of squid observed per hour. This was done for footage taken in the (A) Gulf of California and (B) Monterey Basin. The mean, mode, and median number of squid per hour was shallower in the Gulf of California.

Figure 2:

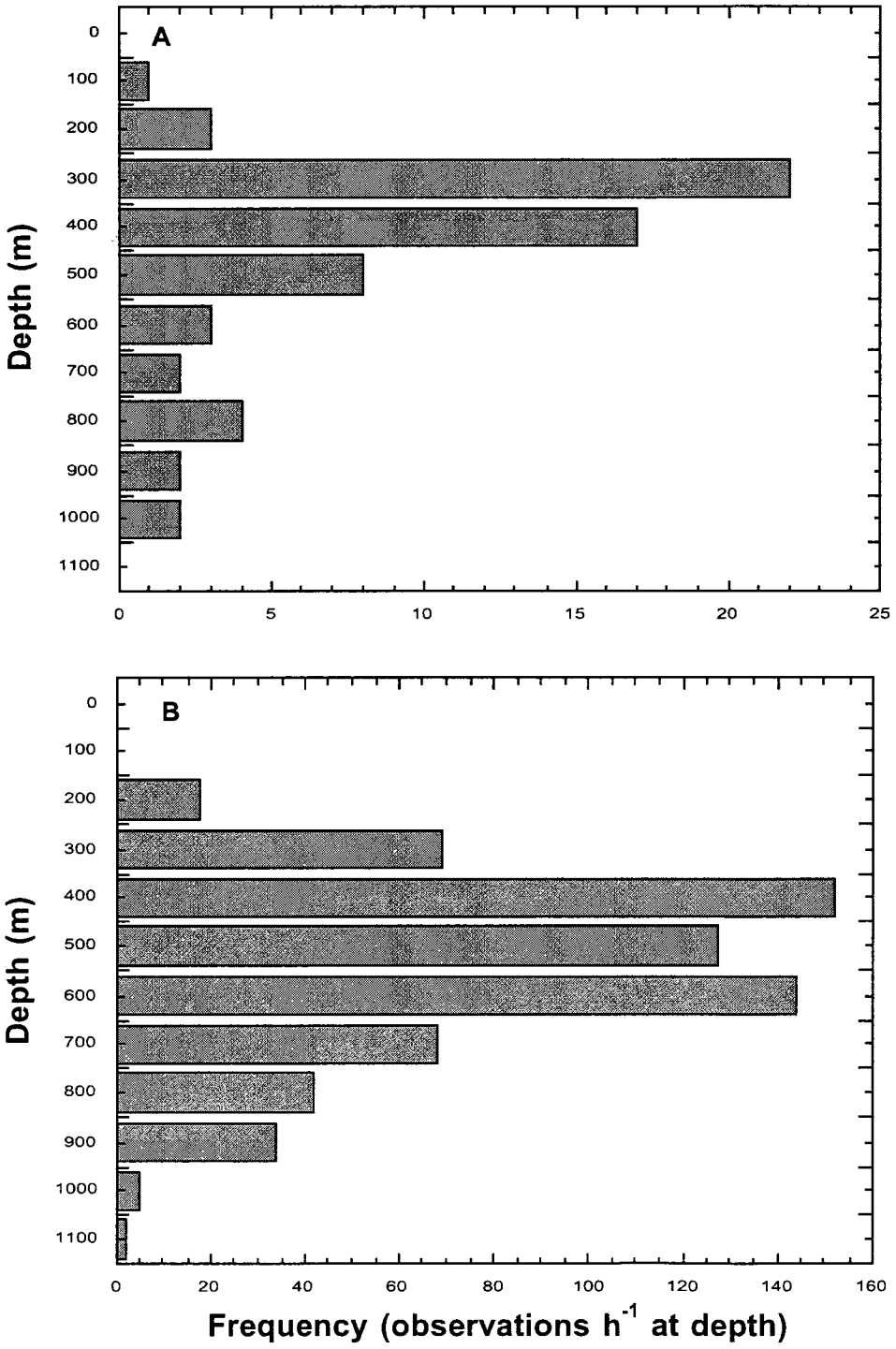

A
B
Depth (m)
0
100
200
300
400
500
600
700
800
900
1000
1100
0
5
10
15
20
25
0
20
40
60
80
100
120
140
160
Frequency (observations h-1 at depth)

Figure 3:
An example of a typical respiration run for *Dosidicus gigas* in a closed respirometer. The individual in this example weight 7 kg. Critical partial pressure was observed at 580 minutes. The squid died after 2 hours of exposre to complete anoxia as indicated by cessation of mantle contractions.

Figure 3:

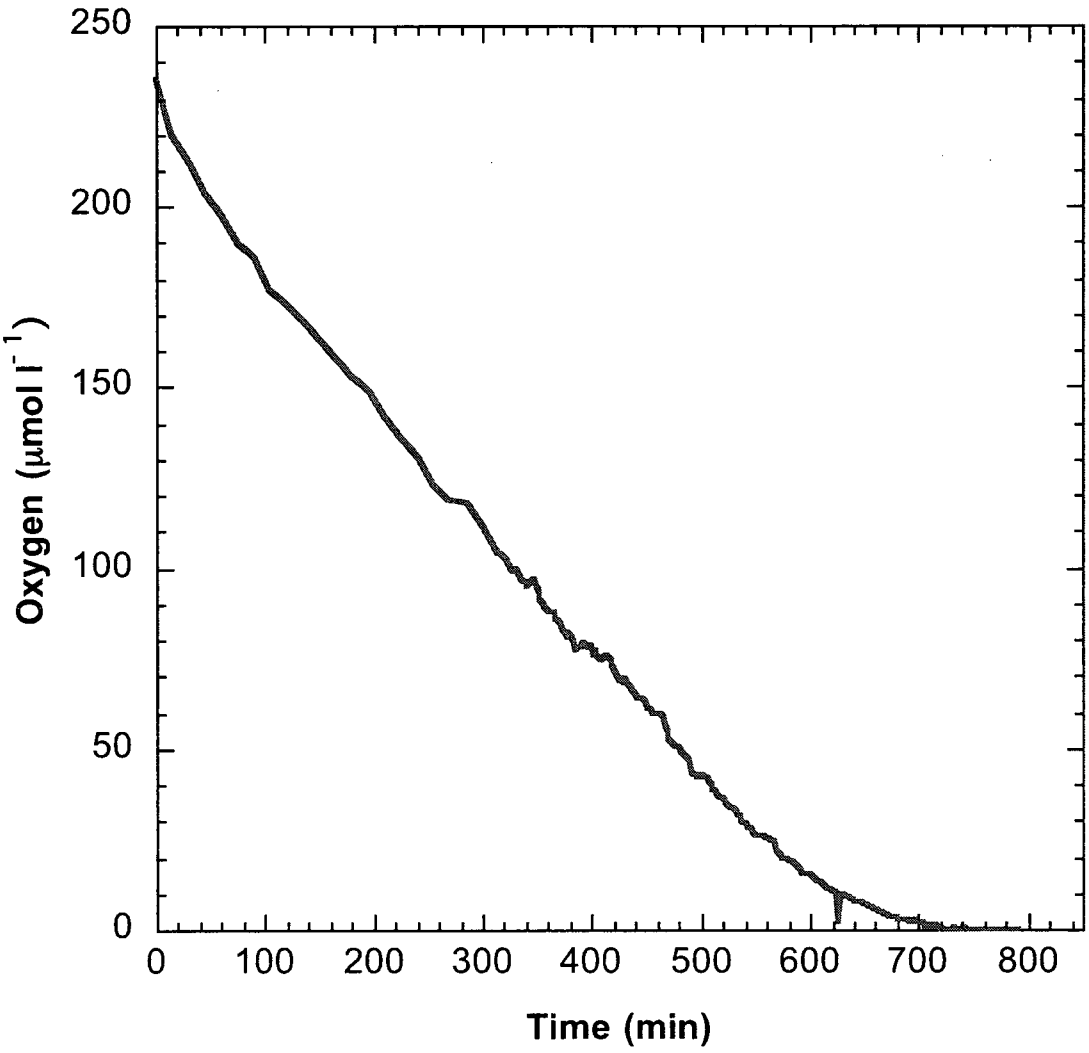

250
200
150
100
50
0
Oxygen (µmol l-1)
0
100
200
300
400
500
600
700
800
Time (min)

Figure 4:
Mean oxygen consumption rates for squid at 10°C (n = 6) and 20°C (n = 5) as a function of the oxygen partial pressure in a closed respiration chamber (A). The critcial oxygen partial pressure, where oxygen consumption is no longer maintained independent of oxygen parital pressure in the chamber, was 1.6 pKa and 3.8 pKA respectively. Oxygen (B) and temperature (C) profiles were recorded via CTD casts. The critical partial pressure is marked by black vertical lines (B). The depth where 10°C and 20°C occur is marked at the intersection of data with the black vertical lines (C). Casts were taken in the Guaymus basin (black squares), Santa Rosalia (grey squares), Carmin Basin (open squares), and Monterey Basin (open circles).

Figure 4:

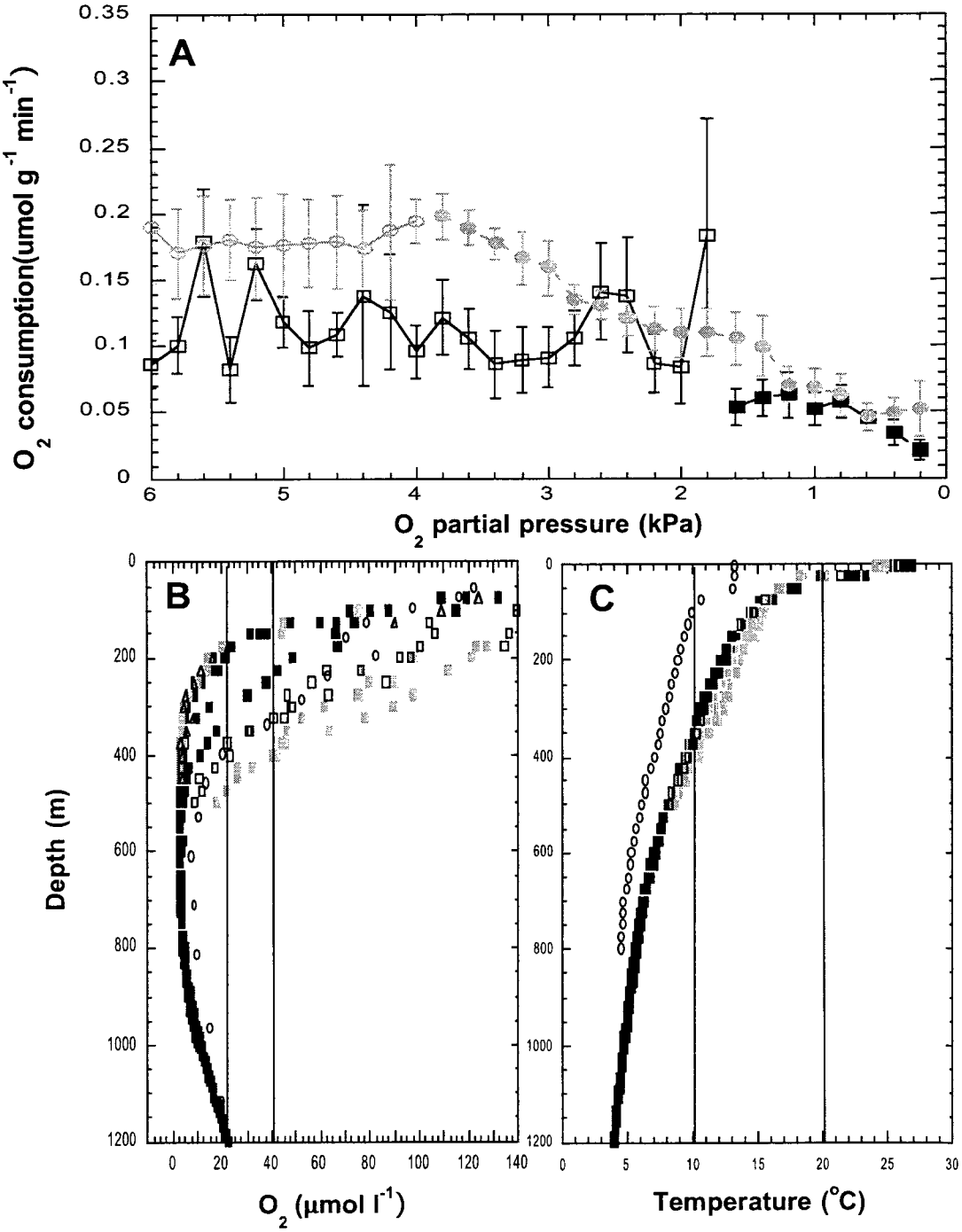

A
B
C
O2 consumption(umol g-1 min-1)
O2 partial pressure (kPa)
Depth (m)
O2 (µmol l-1)
Temperature (°C)

Figure 5:
A) Routine metabolic rate (RMR) as a function of body mass (M) across the full size range of animals captured ($RMR = 11.18M^{-0.108\pm0.02}$), $r^2 = 0.43$, $N = 90$). Measurements were taken at 10°C or temperature corrected from 20°C using a Q_{10} of 2.1. The standard error of the slope did not include quarter power. B) Citrate synthase values, used here as a proxy for RMR (Seibel 2007), decrease with increasing mass (M) according to $CS = 2.39M^{-0.19\pm0.03}$, $r^2 = 0.84$, $N = 70$. Samples are included from the Gulf of California (solid squares) and Monterey Basin (open squares). Citrate synthase activities were measured at 20°C.

Figure 5:

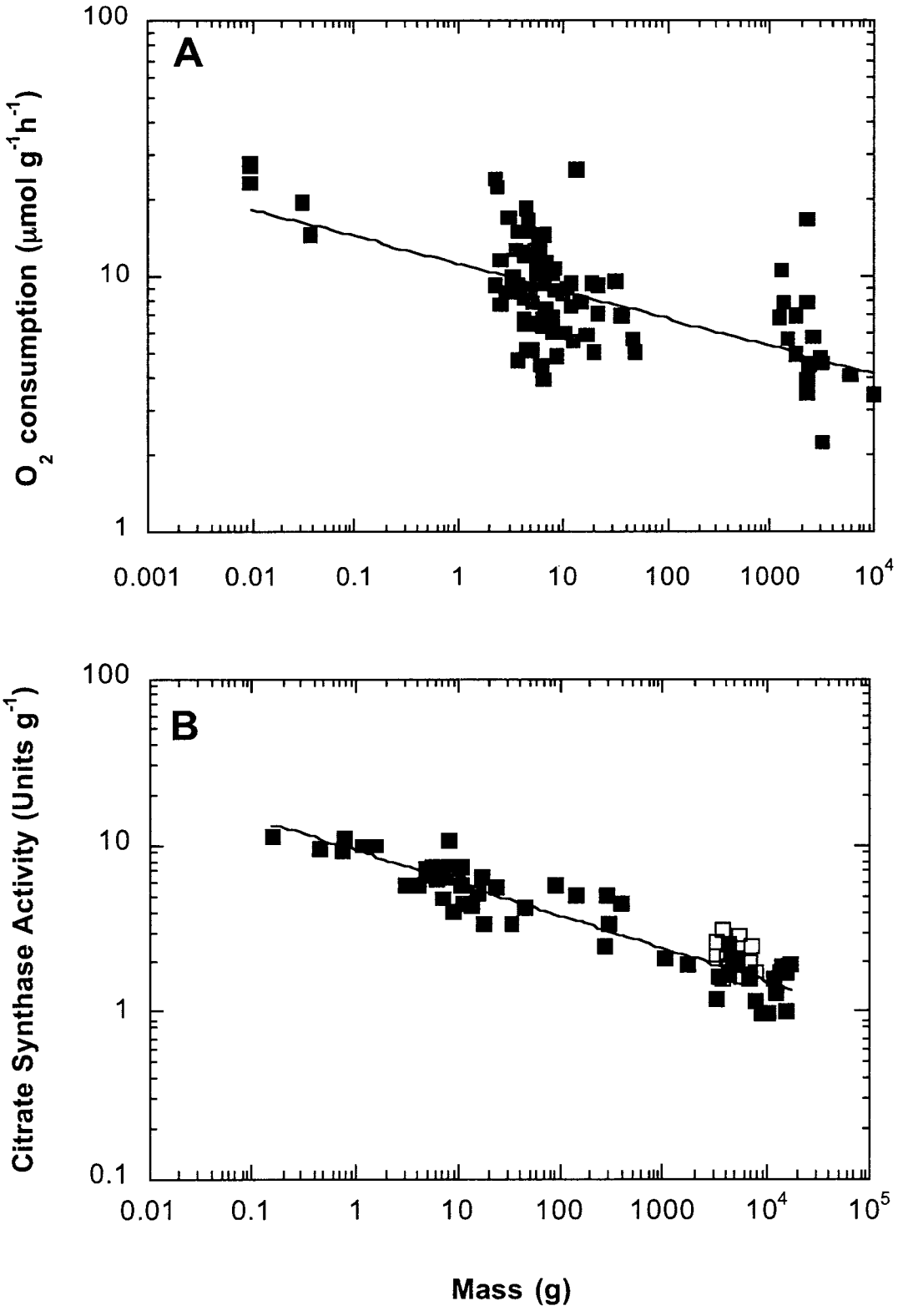

A
100
10
1
O_2 consumption (μmol $g^{-1}h^{-1}$)
0.001
0.01
0.1
1
10
100
1000
10^4
B
100
10
1
0.1
Citrate Synthase Activity (Units g^{-1})
0.01
0.1
1
10
100
1000
10^4
10^5
Mass (g)

Figure 6:
The mean oxygen critical oxygen partial pressure as a function of the minimum environmental oxygen partial pressure encountered in the respective habitats of *Dosidicus gigas* (at 10 and 20°C), experienced during day (open square) night (filled square) and a variety of other cephalopods (circles), pelagic crustaceans (dark triangles) and day/night critical partial of *Vampyroteuthis infernalis, (*open circle), *Histioteuthis heteropsis* (X), *Japetella heathi* (+), *Japetella diaphana* (open diamond), *Bathyteuthis abyssicola* (square with diagonal) *Gonatus onyx* (square with +), *Abraliopsis felis* (square with cross) and *Abraliopsis pacificus* (left pointing triangle), *Nautilus pompilius* (right pointed triangle), *Octopus californicus* (circle with dot). Data are from Seibel et al., 1997, Hunt and Seibel, 2000, Staples et al., 2000, and Childress and Seibel, 1998. As many of the represented species experience the same geographic minimum partial pressure, several data points are hidden, while 53 points are visible, there are a total of 74 points plotted. The diagonal line (unity line) shows the point where P_{crit} matches the environmental oxygen partial pressures. Organisms to the right of the line are in environments where their P_{crit} is lower then environmental oxygen partial pressure. Organisms to the left have P_{crit} above environmental oxygen partial pressure and are unable to maintain aerobic metabolic rates. While in the OML, *D. gigas* (open square) cannot maintain routine aerobic metabolic rates.

Figure 6:

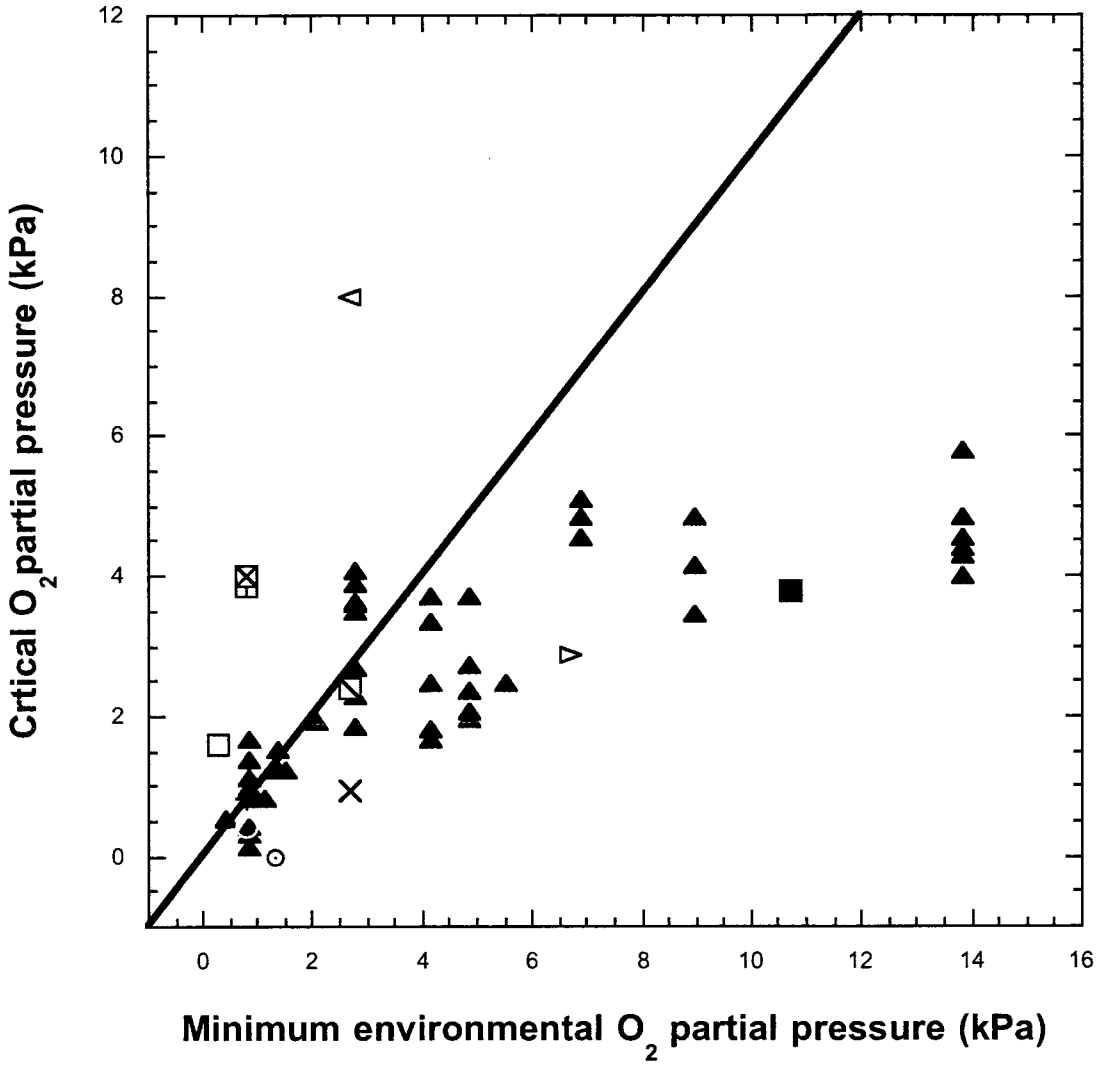

Crtical O2 partial pressure (kPa)
Minimum environmental O2 partial pressure (kPa)
0
2
4
6
8
10
12
14
16

Figure 7:
Preliminary estimates of cost of transport at 0.25 m sec-1 are presented as a function of swimming speed (A) and body mass (B). A) The relationship between speed and COT is parabolic, reflecting costs associated with support in the water column at low speeds and those associated with overcoming drag at higher speeds. COT was higher for *D. gigas* (Solid circle and arrow) than for other cephalopods. COT data for *Illex, Loligo, Nautilus, Sepia* and *Salmon* were calculated using equations from O'Dor and Webber (1991) and were estimated assuming 20 J per ml O2 consumed. B) The COT as a function of body mass for *D. gigas.*

Figure 7:

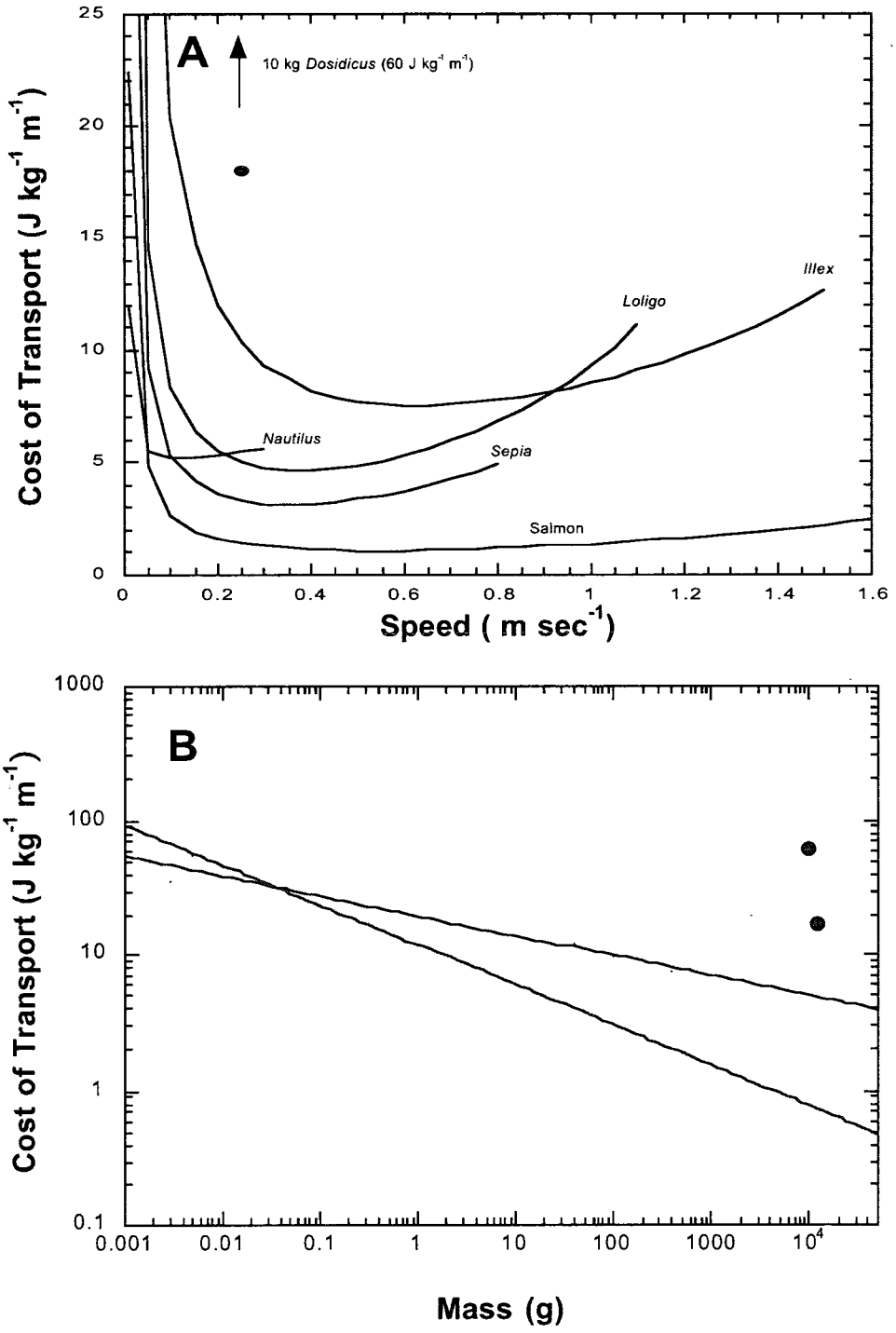
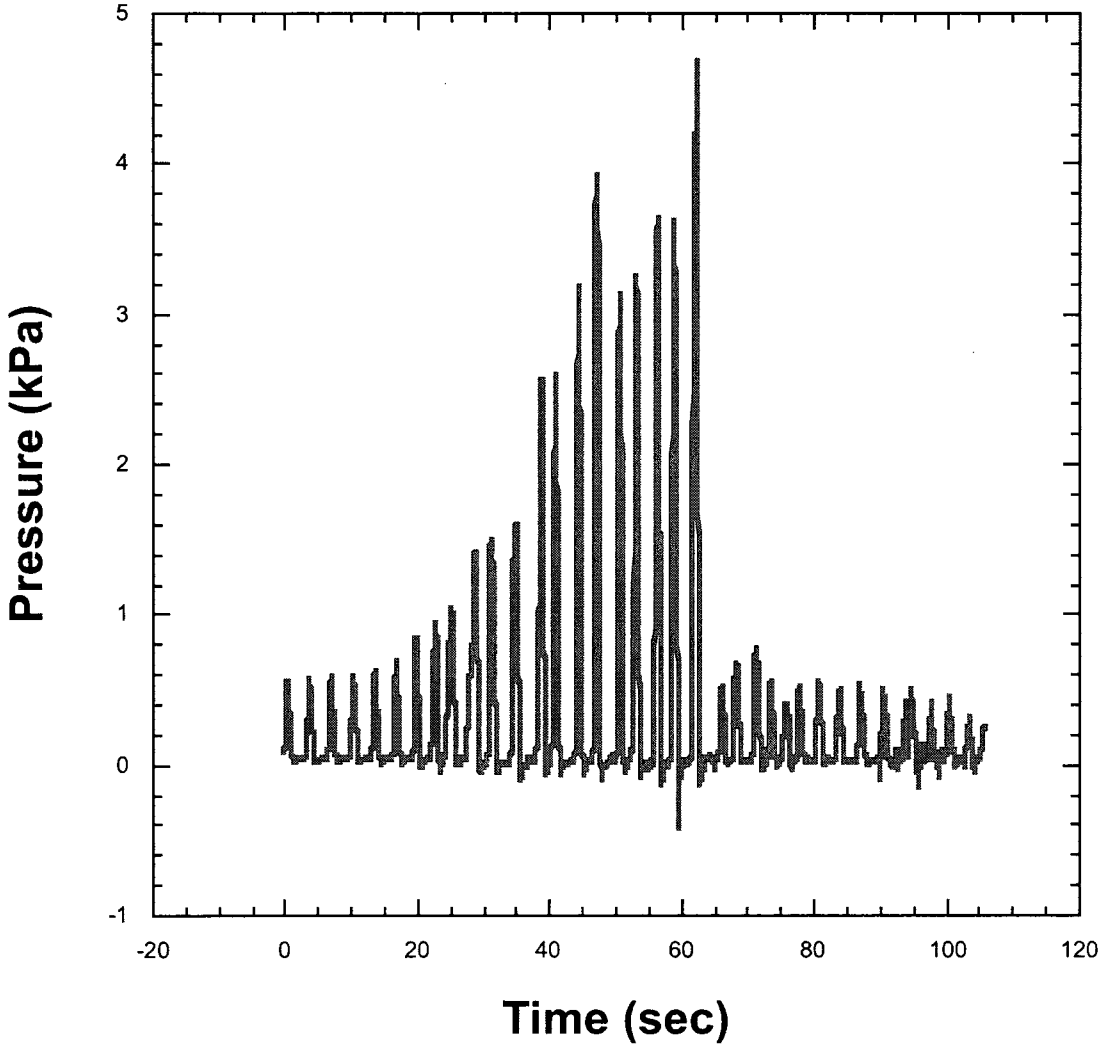

A
10 kg Dosidicus (60 J kg-1 m-1)
Cost of Transport (J kg-1 m-1)
Speed (m sec-1)
Illex
Loligo
Nautilus
Sepia
Salmon
B
Cost of Transport (J kg-1 m-1)
Mass (g)

Figure 8:
Internal mantle pressure of *D. gigas* measured via a internally placed pressure sensor. Measurements were taken as water velocity inside the swim tunnel (Fig. 1) was increased. The pressure was indicative of the force at which water is ejected form the mantle cavity through the funnel opening during active jet propulsion. At rest, cyclic mantle contractions generate a ventilatory water flow past the gills. At sixty seconds, flow rate was stopped and the squid was allowed to rest on the bottom. The mantle pressures were similar to those reported for *Illex illecebrosus* (Webber and O'Dor 1985). The jet pressure is, in other species, correlated with the rate of oxygen consumption and can be used to estimate field metabolic rates in animals that are tagged and released (Webber and O'Dor 1986). The present measurements are the first attempts to calibrate such pressure tags in *Dosidicus gigas.*

Figure 8:



Chapter 3

An Ethogram of the Jumbo Squid *Dosidicus gigas* (Ommastrephidae) as Observed from Remotely Operated Vehicles

by

Lloyd A. Trueblood[1] ; Brad A. Seibel[2]

In prep for submission to the Biological Bulletin

[1]Ph.D. Candidate, Department of Biology, The University of Rhode Island, Kingston, Ri 02881. Email: Lloyd.trueblood@lasierra.edu

[2]Associate Professor, Department of Biology, The University of Rhode Island, Kingston, RI 02881. Email: Seibel@uri.edu

Abstract

Cephalopods have the ability to rapidly alter their external appearance. They have developed the ability to do this arguable better than any other group of organisms (Hanlon and Messenger 1996). Descriptions of body patterns, which result from components of colors, skin texture, body posture, and locomotion, provide an important level of understanding of squid behavior. Most work in this area has focused on shallow, nearshore species dwelling in complex environments. A commonly posed, but untested, hypothesis states that species from less complex environments will have a more limited body pattern repertoire. Here I test this hypothesis by creating an ethogram of an open ocean species of squid, *Dosidicus gigas*. Observations via remotely operated vehicle (ROV) allowed us to record pattern components and their duration. I found that this species has a catalog of components comparable to cephalopods from the nearshore, with 29 chromatic, 15 postural, and 6 locomotory components. Countershading occupied a major portion of patterns observed, which occurred in some form 68% of all observations. This suggests that crypsis for predator avoidance and prey capture is important for this species. I discuss the functional roles of recorded body patterns in the behavioral ecology of open ocean species.

Introduction

Cephalopods demonstrate some of the most dynamic body coloration, unmatched by any terrestrial animal (Packard and Sanders 1971; Packard and Hochberg 1977; Hanlon and Messenger 1988; Hanlon and Messenger 1996). They have evolved the ability to rapidly change the color and pattern of their skin through the use of neuro-muscularly controlled chromatophores, reflective cells, and polarizing elements (Packard and Sanders 1971; Packard and Hochberg 1977; Cloney and Brocco 1983; Mathger and Hanlon 2007). In some species of octopus and cuttlefish, papillae muscles are able to change the texture of skin, which enables a three-dimensional component to camouflage (Packard and Hochberg 1977). Complete body patterns include components of color, texture, posture, and locomotory behaviors (Hanlon and Messenger 1996).

There has been a substantial amount of work dedicated to cataloging cephalopod body patterns, or ethograms, pioneered by Moynihan and Rodaniche (1982, See Hanlon & Messenger 1996 for review). The use of ethograms has greatly benefited behavioral studies of cephalopods (Moynihan and Rodaniche 1982) , assisted in the development of taxonomic keys (Hanlon and Messenger 1988; Roper and Hochberg 1988), produced comparisons of conserved patterns for phylogenetic studies, and facilitated the identification of subspecies (Moynihan 1985; Hanlon and Messenger 1988; Burghardt and Gittleman 1990).

Most ethograms produced thus far have focused on coastal species that have shown a wide array of body patterns. This has been attributed to the complexity of the

nearshore environment and interactions with other organisms (Moynihan and Rodaniche 1982; Moynihan 1985; Hanlon and Messenger 1996). Much less is known about the diversity and complexity of body patterns in pelagic offshore and deep-sea cephalopods. Due to the challenges of working in these environments, few attempts have been made to establish ethograms for this group. It is presumed that oceanic habitats are relatively simple and that residents will have a comparatively small catalog of body patterns (Moynihan and Rodaniche 1982; Moynihan 1985; Hanlon and Messenger 1996). However, recent work by Bush et al (2009) challenge this presumption. They recorded 59 distinct components in a solitary meso-pelagic squid, which demonstrates complexity equivalent to that found for nearshore species (Hanlon, Smale et al. 1994; Hanlon and Messenger 1996; Hanlon, Maxwell et al. 1999; Hunt, Zeidberg et al. 2000; Jantzen and Havenhand 2003).

Here, I examine the body patterns and behaviors of a nocturnally active, open ocean species of squid, *Dosidicus gigas* (Family Ommastrephidae). This species can be found along the west coast of both North and South America and in the equatorial Pacific (Nesis 1983). The open pelagic realm, where this species is found, has little or no physical refuge (Johnsen 2001; Robison 2004) and is considered to have low complexity (Hanlon and Messenger 1996). Like other ommastrephids, *D. gigas* vertically migrates; spending night hours in surface waters, and descending into deeper waters (>250 m) during daylight hours (Gilly, Markaida et al. 2006). This migratory pattern ensures that *D. gigas* is continuously in a low-light environment (Benoit-Bird, Whitlow et al. 2009). According to Hanlon and Messenger (1996), D. *gigas'* open-ocean environment and nocturnal habits should reduce selection for

diversity and complexity of body patterns. I test this hypothesis by creating an ethogram of body patterns for *D. gigas* from video footage taken via a Remotely Operated Vehicle (ROV) from Monterey Canyon, California and the Gulf of California, Mexico. Additionally, I determined both the frequency and duration of each body pattern and its components.

Methods

Video footage was acquired from the Monterey Bay Aquarium Research Institute (MBARI)'s Video Annotation Reference System (VARS) library system. This library contains 15,000 + hours of video footage and was created using the ROVs *Ventana* and *Tiburon*. The ROV *Ventana* is a 40-hp electro-hydraulic platform capable of dives to 1500 m. Lighting is provided via six 400-W DSPL HID Daylight lamps. The ROV *Tiburon* is an electric platform able to dive to 4000 m and has four 400-W DSPL HMI lights. Both of these vehicles' light arrays provide illumination in the daylight range (5500-5600 °K). Video of each dive is recorded on Panasonic D5 high definition videocassette and/or Sony Digital Beta cam standard definition videocassette (Robison 1992; Robison 2003). As organisms are encountered by the ROV, they are annotated using VARS and synchronized with hydrographic parameters (Robison 1992). Using the catalog created via the VARS system, I observed every catalogued instance of *Dosidicus gigas* recorded from the start of the program in 1988 until 2004. Footage was uploaded via a Sony HDR-HC7 video camera onto a Mac Book using Final Cut Express (Apple Computers). Final Cut Express was used to convert the video sequences into bitmap files. Sequences of interest could then be observed one frame at a time. Each sequence was viewed multiple times.

Components were described and recorded as chromatic (color display), postural (positioning of body, limbs and fins), or locomotor (means of movement). Components were classified using established nomenclature for other cephalopod species (Hanlon 1982; Hanlon 1988; Mauris 1989; Porteiro, Martins et al. 1990; Hanlon, Smale et al. 1994; Roper and Vecchione 1997; Hanlon, Maxwell et al. 1999;

Hunt, Zeidberg et al. 2000; Hunt and Seibel 2000; Jantzen and Havenhand 2003; Kubodera, Koyama et al. 2007; Bush, Robison et al. 2009). I used naming guidelines proposed by Hanlon and Messenger (1996) and Hunt (2000) when new components were observed.

Components that were observed less than 5 times over the duration of the study were discarded from statistical analyses. Within component classes (i.e. chromatographic, postural or locomotor) means and standard error (SE) of duration were calculated for each component. An ANOVA with a Newman-Keuls post-hoc test for multiple comparisons was used to determine if there were significant differences ($p < 0.05$) amongst the component means. In each class, the total duration (in seconds) was calculated for each component. This was then converted to % of total time observed.

There is potential for substantial confusion when creating terminology for ethograms of cephalopods. As outlined in Hunt et al (2000), there is inconsistency in the use of the terms "pattern" and "display". Pattern has been used largely for cryptic behaviors, and display for communication behaviors. However, these terms are regularly interchanged and their definition ignored. (Moynihan 1985; Hanlon and Messenger 1996; Hanlon, Maxwell et al. 1999). Here, I refer to all combined components as patterns. Also, there are examples where a component's function is used as a descriptor (such as chasing, jockeying and parrying). Here I follow the naming methods of Hunt et al (2000) by describing the action/appearance of the component being observed, not its function. This allows for simpler comparison of components and patterns between species.

The majority of ethogram studies have been produced via scuba diving. This method provides the distinct advantage of easily following study subjects through the duration of a behavior or set of behaviors. This study was constructed solely from footage taken by ROV. Subjects would swim in and out of the field of view, often during a behavior. Therefore, our duration and prevalence data must be considered estimates; they may not represent the full duration of a behavior. However, the use of the ROV allows us to describe body patterns of organisms that are beyond the depth limits of SCUBA diving. As noted in Vecchione and Roper (1991), critics of studies from submersibles argue that observed behaviors are influenced by the ROV's presence, rendering the studies invalid. However, a loud, well-lit submersible could provoke defensive postures similar to the response when a predator is detected. Minimally, this would allow the characterization of defensive body patterns. While these were observed, much of the video footage actually showed D. gigas foraging and capturing prey in the lights, largely ignoring the ROV. Though these behaviors are surely part of this species' natural catalog, it must be assumed the ROV had some effect on behavior.

Results:

From January 1998 through March 2003, 319 *D. gigas* were observed during 34 ROV dives. I observed 50 distinct body pattern components consisting of 14 light chromatic, 15 dark chromatic, 15 postural, and 6 locomotory components (Table 1, Fig. 1).

Ethogram

Descriptions of each component are listed in Table 1 and Appendix 1, chromatic and postural components can be seen in Figure 1 and 2 respectively.

Duration of behaviors

All components observed had a mean duration < 60 s (Table 1) and are classified as "acute" according to methods outlined in Hanlon and Messenger (1996). Only three components had durations > 10 s. This lends support to the three-tier system suggested by Jantzen and Havenhand (2003) of acute (≤ 10 s), medium acute (11-60 s), and chronic (> 60 s). Such a system allows greater resolution of differences when comparing duration of components between species. One-way ANOVAs with Newman-Keuls post hoc test showed there were significant differences in mean duration in each of the component classes. For chromatic components ($F_{29, 596} = 5.187$, $p > 0.0001$) Countershading pale arm tips, countershading pale fin edges, and Red with black dorsal surfaces were all significantly longer than pale body dark fin edges and arms, pale dorsal stripe, and pale body dark head and arms (Table 1). Additionally, countershading dark head and arms was found to be significantly longer than pale body dark head and arms (Table 1). While an ANOVA of postural components demonstrated a significant difference of duration among components ($F_{14,}$

$_{755} = 2.343$, $p < 0.01$) there were no individual differences among postures (Table 1). Means of mean locomotion duration were significantly different ($F_{5, 777} = 12.75$, $p<0.0001$), with hovering significantly longer in duration than all other categories. Also, forward glide was shown to be significantly shorter than back jetting or back gliding (Table 1).

The prevalence of time (% of total time observed) of each component was examined within its component class. Countershading occupied the majority with 38.8% of total observational time. None of the remaining chromatic components occupied more than 7% of observed time (Table 1). When all chromatic components that contained and element of countershading were combined, they occupied 68% of observed time. Loose arm tips keeled occupied the greatest amount of observed time in the posture class, displayed 51% of the time. Strike glide was the next most prevalent with 12.7%. The remaining postures did not individually exceed 6% of time observed. Backward jet was the most prevalent locomotor component with 26.3% of observation time. Backward glide (17.6%), backward swim (16.6%), forward glide (18.5%), and hover (18.3%), had similar % of time observed. Forward swim was only observed 2.8% of the time. Forward jet was observed once, lasting 1.4 seconds, but was excluded from analysis.

A rank-order plot of frequency vs. cumulative percentage of time observed shows an asymptote after 46 behaviors (Fig. 3). This suggests that the data I have represents a complete catalog of behaviors according to the methods described in (Lehner 1996). It is important to note that all observations presented were taken during daylight hours when the squid were at depths below 150 m. This catalog is

only complete within this context. Personal observations via SCUBA of *D. gigas* in surface waters contained components not observed from the ROV, as discussed below.

Discussion

The diversity of total components observed for *D. gigas* is comparable to those described in other squid ethograms (Table 2). Of these ethograms, all species except for one (*Octopoteuthis deletron*) are from near-shore, complex environments. Hanlon and Messenger (1996) postulated that as habitat complexity increases, body pattern richness should increase in cephalopods. Using chromatic components as an example, they showed coral/rock reef and kelp dwelling species being most diverse (35-12 components) social near reef squid next (23-13 components) followed by murky habitat (15-13 components) and lastly nocturnally active species (14-10 components). Recently Bush et al (2009) found *Octopoteuthis deletron*, a solitary midwater luminescent species of squid, had 17 chromatic components, comparable to reef and social species. I have recorded 29 chromatic components in *D. gigas*, which is also comparable to reef and social species.

As discussed by Bush et al (2009), it is unknown why a squid living in a simple environment would posses such a diversity of body patterns. They suggest that camouflage, interspecific communication to attract prey or deter predators, intraspecific communication for mate selection and species recognition, and several non-communicative options are selective pressures. While *D. gigas* does not spend much time below the photic zone, it does stay in a low light environment, descending to deep water during daylight hours and returning to surface waters at night (Gilly, Markaida et al. 2006; Benoit-Bird, Whitlow et al. 2009). They have visual acuity well suited for observing and capturing prey in this environment (Sweeney, Haddock et al. 2007). The tendency of this species to stay in a low light environment in the open

ocean suggests that selective pressure for a diverse catalog would be low. I discuss our results from this perspective.

Implied in the hypothesis that the complexity of the environment drives the diversity of body patterns is that the primary function of body patterns is crypsis. An organism living in an environment with a diversity of substrates would be well served to have a broad catalog of body patterns. Cuttlefish, for example, rely on three camouflage themes (uniform, mottle, and disruptive) to mask themselves. Variation in color components of these three themes allows them to produce 20-50 distinct chromatic patterns (Hanlon and Messenger 1988). In the pelagic realm, body patterns which focus on transparency, silvering, red coloration, and countershading provide good camouflage (Hanlon and Messenger 1996; Johnsen 2001). Countershading plays a large role in cephalopod crypsis, with several groups, including ommastrephids, having greater densities of chromatophores on their dorsal surfaces to support this pattern (Ferguson and Messenger 1991). Countershading masks aquatic organisms' shadow, when viewed from below, and hides its outline when viewed from above (Thayer 1909; Cott 1940; Ruxton, Speed et al. 2004; Rowland 2009). Hanlon and Messenger (1996) postulated that ommastrephids would have chromatic displays that consisted largely of countershading. Our data support this; of the 29 chromatic components I observed, 15 had some portion of the body countershaded (Table 1, Fig. 1). For the total time observed, 68.6% involved countershading (Table 1). I observed inverse countershading presumably in response to the lighting of the ROV. Inverse countershading of this nature strongly suggests that *D. gigas* is able to detect the direction of light and adjust chromatophores to provide cryptic patterns.

Crypsis in *D. gigas* supports both predator avoidance and prey capture. Several visually based predators such as tuna, swordfish, sharks, seals, and other cephalopods prey upon *D. gigas* (Klages 1996; Smale 1996; Nigmatullin, Nesis et al. 2001; Markaida and Hochberg 2005; Vetter, Kohin et al. 2008). The ability of *D. gigas* to fade into the visual background via coloration decreases the likelihood of being preyed upon. The primary prey of *D. gigas,* myctophid fishes (Markaida 2006), have the visual acuity to see *D. gigas* in areas of low light. Countershading camouflages *D. gigas* from these prey items, allowing for successful capture.

Coloration and postures also may be used to denote aggression and to intimidate (Hanlon and Messenger 1996). This type of behavior was observed as *D. gigas* interacted with the ROV. When in close proximity to the ROV, individuals often hovered in the J-curl or with stiff arms and no keel. Hovering often included vertical undulations, sometimes in the presence of an ink cloud. Squid would bob their body in and out of the ink cloud to observe the ROV. The distinct red with black dorsal surfaces color pattern was typically associated as well. This posture and coloration are visually aggressive in appearance and appear to be used to ward off potential predators.

Aggressive body patterns that stun or shock prey increase the ability to capture prey successfully. The deep-sea squid *Taningia danae,* swiftly approaches prey, and once in close proximity, rapidly extends all arms radially, emitting short flashes from the arm tips (Kubodera, Koyama et al. 2007). This display may serve to disorient prey at the moment of strike, allowing *Taningia danae* to then grab prey items with its arms. A comparable display is seen in the "arms only strike" of *Illex illecebrosus*

(Foyle and O'Dor 1988) and *D. gigas*. While *D. gigas* does not have light emitting organs on its arm tips, the rapid spreading of arms is accompanied by pale coloration of the arm surfaces. This motion and coloration may shock prey items and allow them to be captured.

A combination of coloration and postures is used by *D. gigas* to mask itself and disorient prey during tentacle strikes. As *D. gigas* approaches prey a "strike glide" posture is assumed while forward gliding. This posture diminishes the likelihood of visually stimulating prey as a result of its round cross section, and by increasing the portion of the body anterior of the widest dorso-ventral portion of the body (Trueblood et al., in submission). The bilaterally pale and dark color component is often presented once prey are in close proximity. The contrast of this pattern may serve to confuse prey items. Also, as the squid initiates a strike, three arm pairs are rapidly extended, one vertically and two laterally, while the remaining pair is used to guide the tentacles. The rapid spreading of these arm pairs is visually similar to the arms only strike, and motion of which may confuse prey while the tentacles are extended to grab them. Similar strike patterns have been seen in nearshore squid *Doryteuthis plei* (Kier and Leeuwen 1997) and *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000).

Intraspecific communication during mating, schooling and shoaling has been the focus of many cephalopod ethograms (Hanlon 1988; Hanlon, Smale et al. 1994; Hanlon, Maxwell et al. 1999; Hunt, Zeidberg et al. 2000; Jantzen and Havenhand 2003). During our observations of *D. gigas*, I did not observe any mating activities. While there were numerous cases of multiple squid in view at once, none appeared

organized as a school. Typically, individuals were observed foraging for prey in the light of the ROV, several body lengths or more from each other. They appeared to ignore local conspecifics during our observations, thus I cannot comment on the importance of intraspecific communication in *D. gigas*. It is important to note that all of our observations were taken during daylight hours, when the squid have descended into the oxygen minimum layer. Observations via SCUBA at night in surface waters have shown schooling behavior, and color components not observed in this study, such as very rapid repeated color change from pale to red, or pale to dark (Trueblood personal observation). Observations of *D. gigas* in this environment, and during mating, would surely expand the catalog of observed body patterns.

While crypsis, predator- prey interactions, and intra-specific communication support the need for body patterns observed in *D. gigas*, there may be non-communicative selection for body patterns pelagic cephalopods as well. Bush et al., (2009) discussed locomotor efficiency, ontogeny, and phylogeny as potential causes for diversity of components in *O. deletron*. While I did not observe color patterns associated with a particular locomotive activity as suggested by Hunt (1996) and Huffard (2006), several postural components were associated with locomotion. Hunt (1996) hypothesized that many of the postures observed in deep-sea cephalopods are beneficial for locomotory efficiency by decreasing drag. I observed several postures of *D. gigas* that could benefit the hydrodynamics of the organism. The arm keels were often extended during jetting, as if the were being used as a foil or control surface. The dorsal arm arch was used in a similar manner, providing a keel that was positioned dorso-ventrally, like the tail of an airplane. The stiff arms, and strike glide

postures could benefit as well by reducing the drag of the arms and fully extended fins. These types of postures may increase maneuverability and decrease drag. I did not observe juveniles in this study, so cannot comment on the role of ontogeny in body patterns. I have shown functionality of these components for prey capture, predator avoidance and swimming efficiency.

In conclusion, I have shown that *D. gigas,* an open ocean species, has a diversity of body patterns comparable to cephalopods from well-lit nearshore environments. These patterns appear to be used for crypsis during prey capture and predator avoidance. There is potential for these patterns to be used intra-specifically, but I did not observe close conspecific proximity in this study. The hypothesis that complex environments increase the diversity of components is not supported by our data. There must be another underlying mechanism driving selection for complexity in the open-ocean. A comparative study of the behavior of *D. gigas* in surface waters would likely expand the already broad catalog reported here.

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Table 1:
Body pattern components, mean duration (**MD**), percantage of time observed (**P**), and description of body pattern compenents for the squid *Dosidicus gigas*: **n** is the number of times each component was observed.

Table 1:

Component	n	MD	P	Description
Light Chromatic:				
1. Pale	68	2.7	6.5	All chromatophores are contracted giving the entire squid a pale or pale coloration
2. Pale lateral stripe	38	4.3	5.6	Body dark or countershaded, pale stripe on lateral mantle, occasionally on outer arms
3. CS pale arms	16	2.3	1.3	Mantle and head countershaded, arms varying amounts of pale
4. CS pale arm tips	14	9.6	4.7	Mantle, head, and most of the arms countershaded, arm tips pale
5. CS pale outer arms	13	5.5	2.5	Mantle, head, and arm pair I, III, and IV countershaded Arm pair II pale
6. CS pale ventral shield	13	5.2	2.3	Countershading,ventral mantle below fins has triangular-shaped pale area
7. Pale, red fins	13	2.6	1.2	Arms, head, and mantle pale, fins and dorsal mantle between the fins red
8. Pale, dark fin edges and arms	9	1.4	0.4	Mantle, head, and fins pale coloration, edges of the fins have dark band, arms dark
9. Pale, dark fins, head and arms	8	4.2	1.2	Mantle pale, fins, head, and arms dark.
10. Pale dorsal stripe	7	1.5	0.4	Mantle dark or countershaded, thin pale stripe anterior/posterior dorsal mantle
11. CS pale fins	6	3.7	0.8	Mantle, head, and arms countershaded, fins pale
12. CS pale fin edges	6	12.4	2.6	Mantle, head and arms countershaded, fin edge pale,
13. CS, pale ventral stripe	5	4.8	0.8	Mantle countershaded, thin pale stripe on the ventral portion directly below the fins
14. Pale, dark head and arms	5	1.5	0.3	Mantle and fins pale, arms and head are dark
Dark Chromatic:				
15. CS	209	5.3	38	Dorsal mantle, head, and arms dark, fading to pale on ventral surfaces
16. Dark	45	2.6	4.1	Entire animal has dark red/brown/black color
17. Bilaterally pale and dark	40	2.1	3.0	Entire body of the animal is divided laterally, one side pale and the other dark
18. CS dark head	17	8.1	4.8	Countershaded with dark band on the head and over the eyes
19. Mottle	17	3.2	1.9	Pale and red/dark splotches create an irregular pattern on the whole body
20. CS dark head and arms	13	8.2	3.7	Head and arms are dark, mantle is countershaded form
21. CS dark head pale arms	11	5.6	2.2	Mantle countershaded but the head has a dark coloration, and arms are pale
22. Red with black dorsal surfaces	11	11.2	4.3	Dorsal arms, head, mantle, and fins are black, ventral surfaces are red
23. CS dark arms	10	5.8	2.0	Mantle and head countershaded, arms have dark coloration
24. Red	10	3.9	1.4	Entire animal uniformly bright crimson red, not black or brown associated with dark
25. Inverse CS	6	3.9	0.6	Dorsal surfaces pale fading to dark on ventral surfaces
26. Sandy	5	3.1	0.5	All body surfaces have grainy mix of dark and pale
27. CS mottle arms	4	8.2	1.1	Mantle and head countershaded, arms mottled
28. Sandy, dark arm stripes	4	4.8	0.7	Mantle, fins, and head sandy, aboral arm surfaces are dark, with pale peripheral edges
29. CS red fins	3	2.8	0.3	Mantle, head, and arms countershaded, fins have red
Postural components:				
1. Arms spread	13	3.9	2.1	Arms stiff, extended, spread out and compressed dorsal ventrally parallel to the fins
2. Dorsal arm arch	42	4.1	6.2	Arm from pair I is elevated dorsally forming a keel and may have a sinusoidal shape
3. Droopy arms	7	3.8	1.0	All arms hang limp, ventrally
4. J-curl	17	3.6	5.8	Arms curled dorsally
5. Loose arm tips, keeled	253	8.7	52	Tufts on aboral arms, extended, trailing arms tips loose, waving during locomotion
6. Loose arm tips, no keel	10	5.2	2.2	Trailing arms tips are seen waving with locomotion
7. Prey handling	47	5.7	5.3	One or more arms are curled, manipulating prey towards mouth
8. Stiff arm tips, no keel	19	2.9	1.2	All arms are pointed in a cone forming a circular shape, tapering to the arm tips
9. Stiff arm tips, keeled	7	1.61	1.1	All arms are pointed in a cone shape with arm keels extended
10. Strike, arms only	13	4.0	0.8	Arms splayed radially while capturing larger prey items
11. Strike glide	141	1.6	13	Arms pointed and stiff, fins each form a hyperbole
12. Strike off center	5	2.3	0.4	Tentacles curving off laterally during prey strike
13. Strike with tentacles	87	2.1	5.5	Arm pair I, II, and III extend radially, pair IV guide tentacles as extend towards prey
14. Trailing tentacles, keeled	10	1.6	3.0	Arms appear stiff, tentacles trail loosely extended beyond arm tips
15. Turning to strike	13	7.6	0.9	Prey is approached tail first, *D. gigas* turns laterally and orient arms towards prey
Locomotion Components				
1. Back glide	111	4.7	18	Fins extended laterally, jet pulses from the siphon move animal posteriorly
2. Back swim	153	3.2	17	Fins undulate dorso-ventrally, move animal posteriorly, occasionally with jetting
3. Back jet	188	4.1	26	Fins wrapped around mantel, jetting from the siphon moves animal posteriorly
4. Forward glide	254	2.1	18	Fins extended laterally, jet pulses from the siphon move animal anteriorly
5. Forward swim	30	2.7	2.8	Fins undulate dorso-ventrally moves animal anteriorly, occasionally with jetting
6. Hover	47	11.4	18	Fins undulating, jetting may occur, anterior-posterior 'bobbing' keeps animal suspended

Table 2:
The number of body pattern components broken down by chromatic, postural and locomotion, reported for several species of squid.

Table 2:

	Chromatic	Postural	Locomotory	Source
Doryteuthis opalescens	13	10	14	Hunt et al., 2000
Doryteuthis pealei	39	5	12	Hanlon et al., 1999
Doryteuthis plei	20			Hanlon and Messenger 1996
Dosidicus gigas	29	15	6	Present Study
Lolliguncula brevis	13			Hanlon and Messenger 1996
Loligo forbesi	17	6	5	Porteiro et al., 1990
Loligo vulgaris	16			Hanlon and Messenger 1996
Loligo vulgaris reynaudii	23	5	9	Hanlon et al., 1994
Octopoteuthis deletron	17	17	22	Bush et al., 2009
Sepioteuthis australis	17	16	15	Jantzen et al., 2003
Sepioteuthis lessoniana	20			Hanlon and Messenger 1996
Sepioteuthis sepioidea	23			Hanlon and Messenger 1996
Ranges:	**13-39**	**5-17**	**5-22**	

Figure 1:
Chromatic components of *Dosidicus gigas*

Figure 1:

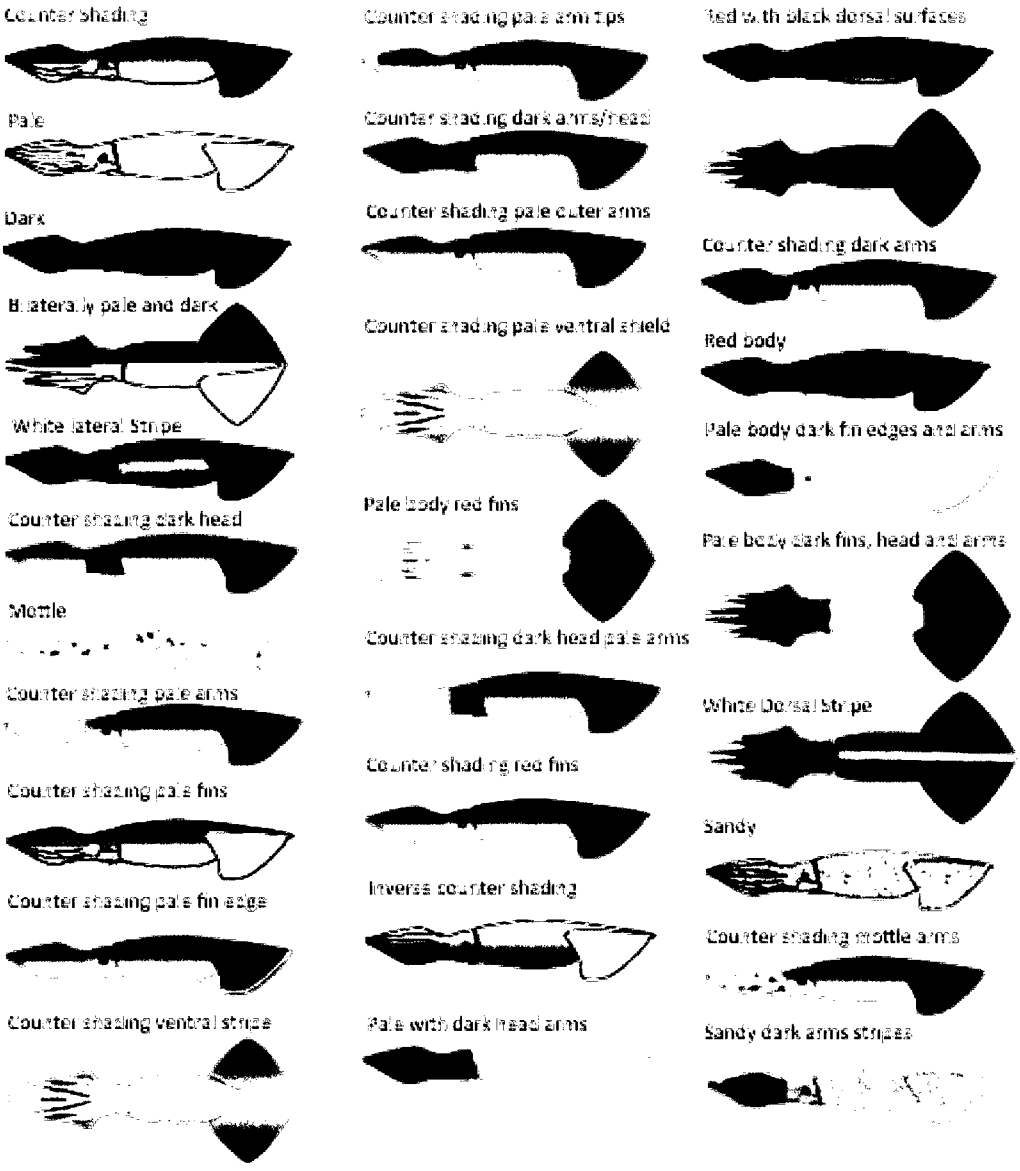

Counter shading
Pale
Dark
Bilaterally pale and dark
White lateral Stripe
Counter shading dark head
Mottle
Counter shading pale arms
Counter shading pale fins
Counter shading pale fin edge
Counter shading ventral stripe
Counter shading pale arm tips
Counter shading dark arms/head
Counter shading pale outer arms
Counter shading pale ventral shield
Pale body red fins
Counter shading dark head pale arms
Counter shading red fins
Inverse counter shading
Pale with dark head arms
Red with black dorsal surfaces
Counter shading dark arms
Red body
Pale body dark fin edges and arms
Pale body dark fins, head and arms
White Dorsal Stripe
Sandy
Counter shading mottle arms
Sandy dark arms stripes

Figure 2:
Postural components of *Dosidicus gigas*: (A) Strike, arms only (B) Arms spread (C) Dorsal arm arch (D) J-curl (E) Droopy arms (F) Strike glide (G) Prey handling (H) Strike with tentacles (I) Stiff arm no keels (J) Trailing tentacles, keeled (K) Stiff arms keeled (L) Loose arms keeled.

Figure 2:

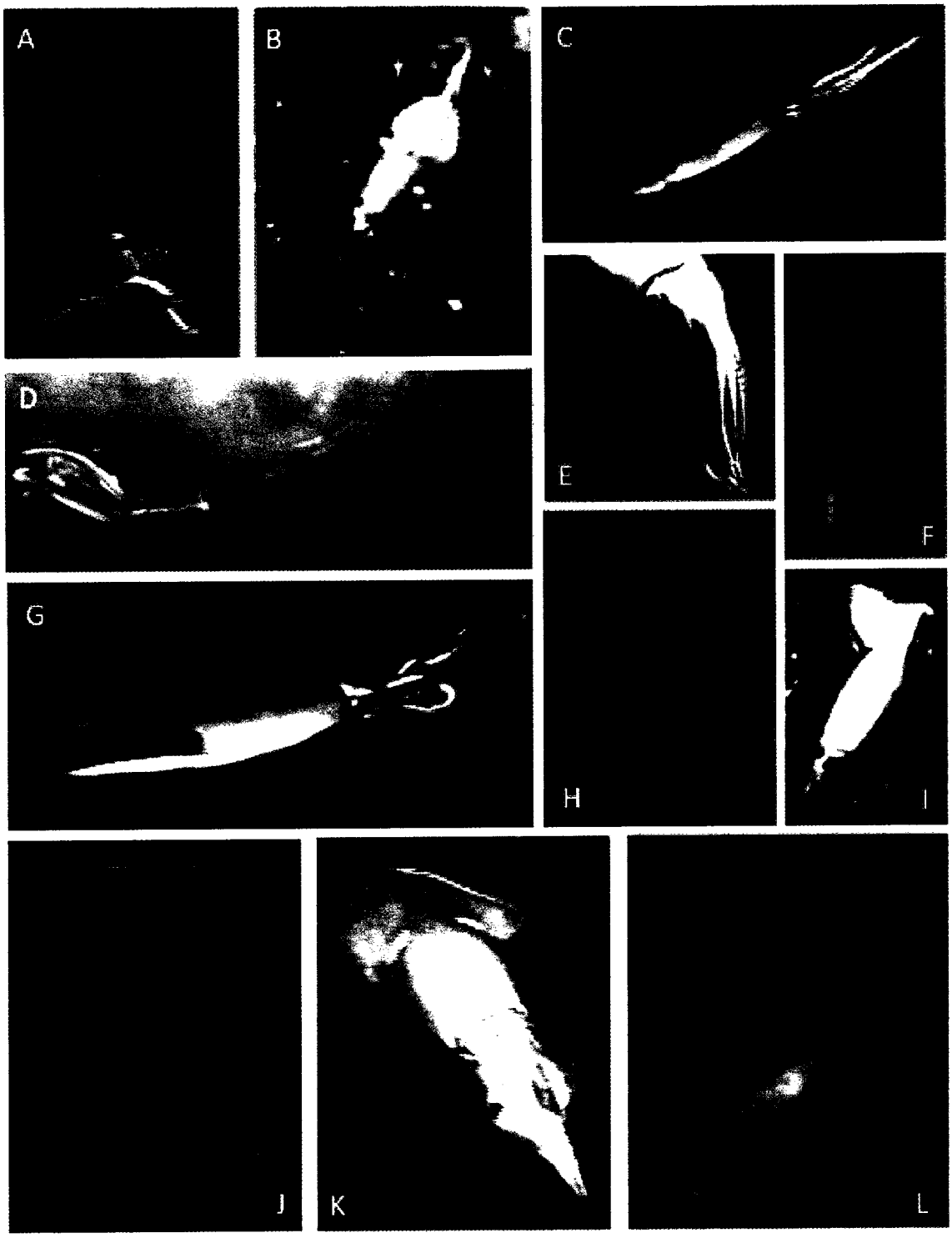
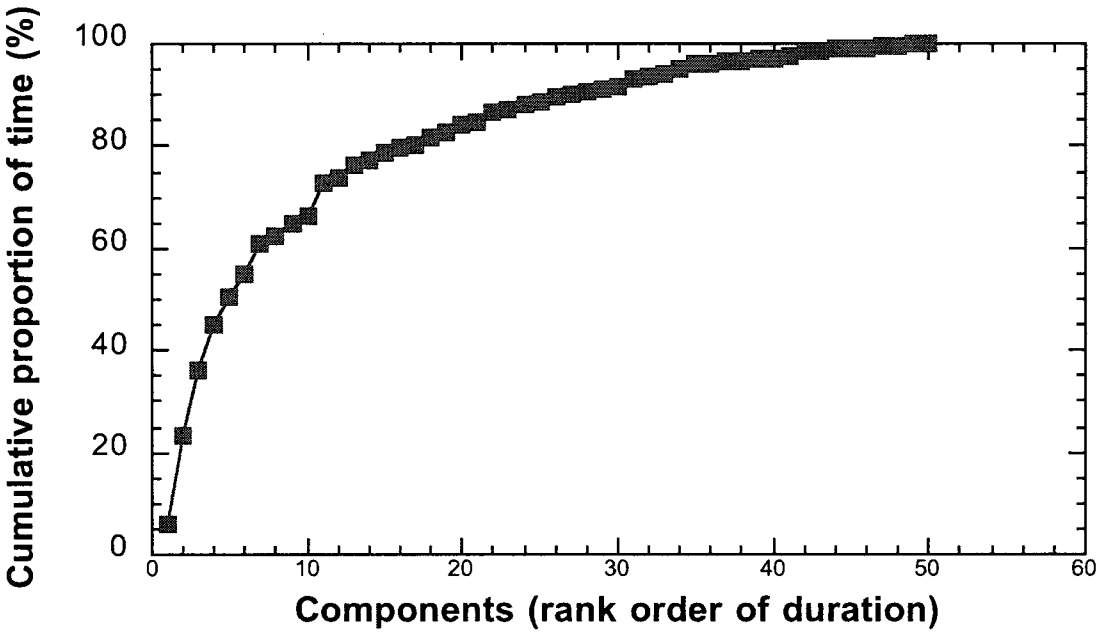


Figure 3:
Rank order of behaviors in cumulative percent of time observed. The X-axis represents a rank order of component by frequency, not assigned component numbers from table 1.

Figure 3:


Cumulative proportion of time (%)
100
80
60
40
20
0
0
10
20
30
40
50
60
Components (rank order of duration)

APPENDIX 1

List of all body pattern components observed for *Dosidicus gigas*. Examples of similar components from other cephalopod species are provided.

Components observed fewer than five times.

Countershading with Dark lateral stripe: A pattern similar to the above-mentioned counter shading with a dark stripe on the lateral mantle running its full length. This is similar to mantle margin stripe reported for *Sepioteuthis australis* (Jantzen and Havenhand) and *Loligo vulgaris* (Hanlon, Smale et al.) and Lateral stripe reported for *Doryteuthis opalescens* (Hunt, Zeidberg et al.).

Countershading with dark center mantle band and dots at fin base: This pattern follows countershading, but there is a dark band just anterior of the fins, with a pair of dark circles near the base of the fins. This is similar to the "lateral mantle spot" reported for *Doryteuthis paelei* (Hanlon, Smale et al.).

Countershading in red: The countershading pattern was followed over all the surfaces of the animal, however instead of fading to pale, the pattern was dark black/brown on the dorsal surfaces and faded to a bright red ventrally.

Dark with pale central arms: the mantle, fins, head and outer arms are dark, with arms 1 and 2 having pale aboral surfaces.

Dark with pale ventral spot: the majority of the animal is in the dark colorations, there is a pale spot just anterior of where fins wrap around mantle while jetting.

Dark Dorsal Stripe: While the majority of the body is pale, there is a dark stripe running anterior/posterior on the dorsal side of the mantle. This is similar to Dark dorsal stripe in *Loligo vulgaris* (Hanlon, Smale et al.) *Doryteuthis paelei* (Hanlon, Maxwell et al.) *Doryteuthis opalescens* (Hunt, Zeidberg et al.) *Alloteuthis*

subulata (Cornwell, Messenger et al.) and *Sepioteuthis australis* (Jantzen and Havenhand) , Dorsal stripe in *Loligo forbesi* (Porteiro, Martins et al.) and *Doryteuthis plei* (Hanlon).

Dark with pale fin edge: The body is dark with the edge of the fin pale. This coloration has been observed in *Octopoteuthis deletron* (Bush, Robison et al.).

Mottle with Ventral stripe and body ring: the majority of the mantle, fins, head, and arms are mottled with a clear stripe running anterior/posterior down the ventral portion of the mantle and a ring around the circumference of the mantle mid way down its length. A ventral mantle stripe has been reported for *Doryteuthis paelei* (Hanlon, Maxwell et al.) and *Loligo vulgaris* (Hanlon, Smale et al.) but these were not associated with a mottled pattern or a mantle ring.

Red dorsal stripe, head and arms: This color pattern was typically associated with individuals while they were rapidly jetting by the ROV. It is marked by a pale body with a bright red stripe that rand the entire dorsal length of the animal, covering mantle, head and arm. While it is similar in shape to the dark dorsal stripe, it is has a very bright red coloration that makes it visually distinct.

Extended suckers: arms are splayed and suckers are visibly extended along the edges of the arms. This behavior was only observed once, but worth noting. It shows the ability to extend suckers in *D. gigas.*

Components observed five or more times

Light Chromatic

Pale: all chromatophores are contracted giving the entire squid a pale or pale coloration. This pattern has been reported as 'clear' for *Alloteuthis subulata* (Cornwell, Messenger et al. 1997) *Doryteuthis plei* (Hanlon 1982) *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999) *Sepioteuthis australis* (Jantzen and Havenhand 2003) *Loligo vulgaris* (Hanlon, Smale et al. 1994) and *Loligo forbesi* (Porteiro, Martins et al. 1990), 'all light" for *Sepiola affinis* (Mauris 1989) and 'Pale' for *Octopoteuthis deletron* (Bush, Robison et al. 2009).

Pale lateral stripe: the majority of the body is dark, or countershaded with a pale stripe along the lateral portion of the mantle, occasionally including the outer arms.

Countershading with pale arms: mantle and head maintain countershading pattern, arms had varying amounts of pale, in some cases extending from the base to the arm tip. This pattern is the same as "pale arms" reported for *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999).

Countershading pale arm tips: mantle, head, and most of the arms follow a countershading pattern. The tips of the arms have a distinct pale coloration.

Countershading pale outer arms: mantle, head, and arm pair I, III, and IV follow a countershading pattern. Arm pair II and associated keels, have pale coloration from the base of the arm extending to the tip.

Countershading with pale ventral shield: the majority of the organism follows the countershading pattern. The area of the ventral mantle, where the fins fold over when they are ventrally wrapped, has a triangular-shaped pale area.

Pale body red fins: arms, head, and mantle are pale. The fins and upper mantle area between the fins have a distinct red coloration. This pattern was seen when the squid was in the J-curl posture and there was ink present. It likely represents a defensive coloration. It is similar to 'dark fins" of *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999) and *Loligo vulgaris* (Hanlon, Smale et al. 1994).

Pale mantle dark fin edges and arms: mantle, head, and fins, have pale coloration. The leading edges of the fins have a dark band, and arms are dark from base to tip.

Pale mantle dark fins, head and arms: mid- mantle is pale; fins, head, and arms are dark.

Pale dorsal stripe: mantle is dark, or in countershading coloration, with a thin distinct stripe running anterior/posterior along the central dorsal region of the mantle.

Countershading pale fins: mantle, head, and arms followed a countershading pattern. The fins are pale in color. This pattern was often seen in, or immediately after, tentacle extension during prey capture.

Countershading pale fin edges: pattern is similar to the pale fin edge seen in *Octopoteuthis deletron* (Bush, Robison et al. 2009), with the fin edge having a distinctly pale color compared to the rest of the body. However, in *D. gigas* instead of the body having a dark coloration, it follows a countershading pattern.

Countershading with ventral stripe: mantle follows countershading pattern with a

thin pale stripe on the ventral portion directly below the fins. It does not extend the full length of the mantle.

Pale with dark head and arms: mantle and fins are pale in color, but arms and head are dark. This is similar to pale tail of *Octopoteuthis deletron* (Bush, Robison et al. 2009).

Dark Chromatic

Countershading: This was the most commonly observed chromatic display in *D gigas* with quite a few variations listed below. The dorsal portion of the mantle, head, and arms are dark in coloration, fading to pale on the ventral surfaces. This is similar to the countershading described for *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999) *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000).

Dark: entire animal has dark red/brown/black coloration. This is the same as all dark reported for *Alloteuthis subulata* (Cornwell, Messenger et al. 1997) *Doryteuthis plei* (Hanlon 1982) *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999) *Sepioteuthis australis* (Jantzen and Havenhand 2003) *Loligo vulgaris* (Hanlon, Smale et al. 1994) *Loligo forbesi* (Porteiro, Martins et al. 1990)and *Sepiola affinis* (Mauris 1989) and Dark for *Octopoteuthis deletron* (Bush, Robison et al. 2009).

Bilaterally pale and dark: entire body of the animal is divided laterally with one side pale and the other dark. This is similar to "all dark unilateral" of *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999) and of *Loligo vulgaris* (Hanlon, Smale et al. 1994).

Countershading with dark head: there is a dark band on the head and over the eyes. This pattern resembles "Shaded eye" of *Doryteuthis pealei* (Hanlon, Maxwell et al.

1999) and *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000) and the "dark head bar" for *Sepiola affinis* (Mauris 1989).

Mottle: Pale and red/dark splotches create an irregular pattern on the whole body. This is identical to the Mottle reported for *Octopoteuthis deletron* (Bush, Robison et al. 2009).

Countershading dark head and arms: area of the head and arms are dark, but the mantle stays in countershading form. The same pattern has been reported as Dark arms/head for *Loligo vulgaris* (Hanlon, Smale et al. 1994) and *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999).

Countershading dark head pale arms: mantle maintains the countershading color pattern, but the head has a dark coloration, and arms are pale. The band on the head is similar to the shaded eye of *Doryteuthis plei* (Hanlon 1982) and Dark head of *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999), except for the color difference between the arms and mantle. The portion of the arms that was pale in color varied from the full arm length to just the arm tips. This is similar to pale arms of *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999).

Red with black dorsal surfaces: This body pattern was only observed while individuals were in the "J-curl posture" (Fig 2 D). The dorsal portions of arms, head, mantle, and fins are black. There is a distinct transition line to red for the remainder of the body.

Countershading dark arms: mantle and head match the fading color scheme of countershading, but the arms have dark coloration.

Red: This is similar to the dark coloration listed above. The entire animal has a uniform color; however, the pigmentation is bright crimson- not black or brown associated with dark.

Inverse countershading: dorsal surfaces of the individual were pale in coloration and faded to dark on the ventral surfaces.

Sandy: all body surfaces have grainy mix of dark and pale.

Countershading with mottle arms: mantle and head maintained countershading pattern. The arms have a pale and red patchwork pattern, like that reported for *Octopoteuthis deletron* (Bush, Robison et al. 2009).

Sandy with dark arm stripes: mantle, fins, and head all follow the sandy pattern. Aboral arm surfaces are dark, with pale peripheral edges causing them to appear as having a large central black stripe.

Countershading red fins: mantle, head, and arms follow countershading pattern. Fins have red coloration.

Postural components

Arms spread: arms are stiff and fully extended. They are spread out and compressed dorsal ventrally to form a flat plane parallel to the fins. This posture has been observed and termed "splayed arms" in *Loligo vulgaris* (Hanlon, Smale et al. 1994), *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999), *Octopoteuthis deletron* (Bush, Robison et al. 2009), *Loligo forbesi* (Porteiro, Martins et al. 1990). Occasionally, this posture was used as a braking mechanism while backward jetting. This is similar to the "arm brake" described for *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000).

Dorsal arm arch: arm an arm from pair I is slightly elevated dorsally and have a

sinusoidal wave shape. When jetting at relatively high velocity, this arch becomes streamlined. It takes on the form of a smaller vertical keel, similar to the lateral arm-keels described below. This may provide some hydrodynamic benefit while jetting at high speeds. Arm arches have been described for several other species of squid (Vecchione and Roper 1991; Hanlon, Maxwell et al. 1999). They have been explained as a means of intimidation during mate selection or used as a lure for prey capture. *Dosidicus gigas*' use of the arm arch is much more subtle. It appears to be associated with locomotion as it was never observed in a squid that was not actively moving.

Droopy arms: all arms hang ventrally as if they are limp. This posture has been observed in *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999) and *Sepioteuthis australis* (Jantzen and Havenhand 2003) and has been termed "downward curl" in *Loligo forbesi* (Porteiro, Martins et al. 1990) and *Doryteuthis opalescens*(Hunt, Zeidberg et al. 2000).

J-curl: arms curled dorsally. This appears to be a defensive posture, often occurring when individuals were in close proximity to the ROV. This behavior was always associated with hovering in *D. gigas*, with fins undulating as if the squid was "flying". The production of an ink cloud was common for individuals in the J-curl and was used similarly to the "ink cocoon" described above. This has been described as a 'J-curl' for *Gonatus onyx* (Hunt and Seibel 2000) and *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000), a 'modified-j' for *Ornithoteuthis antillarum* (Vecchione and Roper 1991) an 'upward curl" for *Sepioteuthis australis* (Jantzen and Havenhand 2003) and as "arms flexed dorsally' *Octopoteuthis megaptera* (Vecchione and Roper 1991).

Loose arm tips keeled: tufts were extended laterally from arms 3 and 4, potentially to provide hydrodynamic stability. Prominent arm keels have been described in *Octopoteuthis deletron* (Bush, Robison et al. 2009).

Loose arm tips not keel: trailing arms tips are seen waving with locomotion. This was typically observed during slow backward swimming and backward jetting. Arms follow the cone shape described below for stiff arms, but the tips are not pointed.

Prey handling: one or more arms are curled, manipulating prey towards mouth.

Stiff arm-tips keeled: all arms are pointed in a cone shape with arm keels extended.

Stiff arm-tips no keel: all arms are pointed in a cone forming a circular shape, tapering to the arm tips. All forms of locomotion were observed as having a stiff arm component. This posture has been described as 'rigid arms' *Sepioteuthis australis* (Jantzen and Havenhand 2003) and 'straight arms' for *Octopoteuthis deletron* (Bush, Robison et al. 2009).

Strike arms only: typically *D. gigas* uses extendable tentacles to reach out and grab small prey items. Occasionally, *D. gigas* was observed to splay arms radially when capturing larger prey items. Tentacles were not used during this means of prey capture. This behavior was observed in *Illex illecebrosus* (Foyle and O'Dor 1988) and *Taningia danae* (Kubodera, Koyama et al. 2007). However *T. danae* does not have tentacles, and uses this as its main means of prey capture. *Dosidicus gigas* likely utilizes this means of capture because it allows 8 grappling surfaces to cling to large prey. This provides better holding power than produced by the clubs at the end of tentacles.

Strike glide: arms are pointed and stiff as prey is approached. Fins each form a hyperbole, and jetting provides propulsion. This was always observed just prior to a tentacle strike. This posture has been observed in *Doryteuthis plei* (Kier and Leeuwen 1997) and is associated with prey strike. It was also observed with *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000) but there was no prey capture correlation stated.

Strike off-center: typically *D. gigas* orients itself such that prey items are directly in front of arm tips. Occasionally, they were observed to strike prey that was off to one side. They curved their arms and tentacles as they extended toward prey.

Strike with tentacles: prey was approached with stiff arms, as strike began. Arms 1 and 2 are lifted dorsally; 3,4, and 5,6 extended laterally; 7,8 pointed at prey- acting as a "guiding trough" for the tentacles as they extend towards prey. This strike behavior has been described for *Doryteuthis plei* (Kier and Leeuwen 1997) and *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000).

Trailing tentacles: arms appear stiff, but tentacles are seen trailing loosely behind arm tips. This occurs while swimming, gliding, or jetting backwards. This behavior has been observed in *Loligo forbesi* (Porteiro, Martins et al. 1990).

Turning to strike: when prey is approached tail first, *D. gigas* turns laterally to orient arms towards prey. This has been described as 'panning" in *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000).

Locomotion

Forward glide: fins are laterally extended, rigid (not flapping) and flat. During prey strikes fins are curled (Fig. 2) and pulses from the siphon provide anterior movement.

Backward glide: similar to the above. Locomotion is the result of slow pulses from the siphon, causing posterior movement. Fins are extended and ridged, acting as control surfaces to maneuver. Both forward and backward glide have been described in *Octopoteuthis deletron* (Bush, Robison et al. 2009) *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000) .

Forward swimming: fins are actively flapping in unison dorso-ventrally, producing low speed swimming. This may be combined with jetting, causing anterior movement. Forward swimming has been seen in *Taningia danae* (Kubodera, Koyama et al. 2007) *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000) *Octopoteuthis deletron* (Bush, Robison et al. 2009) and *Sepioteuthis australis* (Jantzen and Havenhand 2003), and is described as arm first swimming for *Lolliguncula brevis* (Bartol, Mann et al. 2001).

Backward swimming: fins are actively flapping in unison dorso-ventrally, producing low speed swimming. This may be combined with jetting, providing posterior movement. Backward swimming has been described in *Taningia danae* (Kubodera, Koyama et al. 2007) *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000), *Sepioteuthis australis* (Jantzen and Havenhand 2003) *Octopoteuthis deletron* (Bush, Robison et al. 2009), as tail first swimming in *Lolliguncula brevis* (Bartol, Mann et al. 2001), and swimming in *Magnapinnidae sp.* (Vecchione, Roper et al. 2002).

Forward jetting: fins are wrapped tightly around the mantle. Rapid jetting from the siphon provides the sole source of locomotion in the anterior direction. Arm keels were often extended laterally, and occasionally arms 1 and 2 were slightly raised to

form a dorsal keel. It has been described for *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000) and *Octopoteuthis deletron* (Bush, Robison et al. 2009).

Backward jetting: fins are wrapped tightly around the mantel. Rapid jetting from the siphon provides the sole source of locomotion in the posterior direction. Arm keels were often extended laterally, and occasionally arms 1 and 2 were slightly raised to form a dorsal keel. This was the most rapid means of locomotion. It is a common escape mode found in many cephalopods. It has been described as backward jetting for *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000) *Octopoteuthis deletron* (Bush, Robison et al. 2009), and as jetting for *Loligo vulgaris* (Hanlon, Smale et al. 1994) *Sepioteuthis australis* (Jantzen and Havenhand 2003) *Doryteuthis pealei* (Hanlon, Maxwell et al. 1999).

Hovering: individuals maintain their position in the water column, typically oriented with tail up and fins flapping. At times jetting occurred with the siphon moving 180°, resulting in anterior-posterior 'bobbing' movement. This movement was frequently associated with inking with the individual dipping its head in and out of the resulting cloud. This is termed ink cocooning in *Mastigoteuthis hjorti* (Vecchione, Roper et al. 2002). Hovering has been shown for *Sepioteuthis australis* (Jantzen and Havenhand 2003) *Octopoteuthis deletron* (Bush, Robison et al. 2009) *Doryteuthis opalescens* (Hunt, Zeidberg et al. 2000) and *Mastigoteuthis sp.* (Roper and Vecchione 1997).

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