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Metabolism and enzyme activities of hagfish from shallow and deep water of the Pacific Ocean

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ABSTRACT

Although hagfishes are ecologically important members of benthic communities there has been little data available on their metabolism. The oxygen consumption, enzyme activities, and muscle proximate composition of shallow living *Eptatretus stoutii* and deeper living *E. deani* were measured to investigate hagfish metabolism. Very low rates of oxygen consumption and both aerobic and anaerobic enzyme activities in the body musculature confirmed the low metabolism of hagfishes. However, significant variation in oxygen consumption existed. *E. stoutii* had significantly lower rates compared to those of the deeper living *E. deani* and two other shallow living species for which literature data was used. Both species could regulate their oxygen consumption to very low oxygen concentrations. *Epatretus deani*, which lives in an oxygen minimum zone, had a significantly lower critical oxygen tension (0.83 kPa) compared to *E. stoutii* (1.47 kPa). The deeper *E. deani* had greater lipid stores than *E. stoutii* which may reflect its deeper habitat and more sporadic food supply.

1. Introduction

Metabolism is fundamentally important to ecology. It is a process of energy assimilation, transformation, and allocation that strongly influences most of the rate processes of the individual such as resource utilization, growth and reproduction (McNab, 2002; Brown et al., 2004). Through the integration of individuals the dynamics of populations and communities can be linked. Metabolic rates can be used to construct models of the flow of energy and materials in an ecosystem (Childress and Thuesen, 1992; Smith, 1992; Christiansen et al., 2001; Smith et al., 2001). In the deep sea, it is experimentally more difficult to obtain measurements on the metabolic rates of animals than it is in shallower habitats. Studies have assumed that the energetic demands of deeper living organisms can be extrapolated from more easily obtained data on shallower living relatives by using models of the mass and temperature dependence of metabolic rate (Mahaut et al., 1995). Alternatively, a handful of measurements of representative taxa (Smith, 1992; Smith et al., 2001) have also been used for deep-sea species where data were not available.

Hagfishes (order Myxiniformes) are the most ancient fishes, lacking jaws, fins, and gas bladders. They consume infaunal animals and are renowned for their scavenging abilities, burrowing into the carcasses of dead animals on the seafloor (Martini, 1998). They can exude copious amounts of slime which can clog gills and likely explains the general lack of teleost predators (Lim et al., 2006). In some regions, they reach very high abundances (Wakefield, 1990; Catchpole et al., 2006) and they are targeted by commercial fisheries (Barss, 1993; Grant, 2006). Despite their ecological importance, there is very little data on the metabolism of hagfishes.

Metabolism in some animal groups has been shown to decline markedly with increased habitat depth (Seibel and Drazen, 2007). For some taxa, such as pelagic fishes, crustaceans, and cephalopods, declines in metabolic rate (oxygen consumption) are not explained solely by differences in environmental temperature and body mass. This decrease in metabolic rate can be 1–2 orders of magnitude greater than rates expected after mass and temperature correction. Interestingly, these declines are matched by declines in the activities of metabolic enzymes (Childress and Somero, 1979; Seibel et al., 2000). A possible explanation for these declines is the visual interaction hypothesis (VIH; Childress, 1995; Seibel and Drazen, 2007) that suggests that in the dimly lit and sparsely populated deep-sea, predators and prey do not interact as frequently or over as large a distance, relaxing the need for locomotory capacity which reduces metabolism. In brightly lit surface waters animals are able to detect both predators and prey at a great distance (Lythgoe, 1988) and must have higher metabolic rates and greater locomotory capabilities for long chases or escapes.

Unlike almost all other fishes, hagfishes are functionally blind (Martini, 1998), even species living in very shallow water. This characteristic makes them interesting subjects to test the VIH, which predicts no difference in metabolic rates between shallow and deep living species for non-visually cued animals. Surprisingly, the single

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measurement of metabolic rate in the deep-sea hagfish, *Eptatretus deani* (Smith and Hessler, 1974), suggested a lower metabolic rate than in shallower living hagfish species (Munz and Morris, 1965; Steffensen et al., 1984; Kench, 1989). It is clear that additional data on hagfish metabolism would contribute to evaluating the VIH in the context on non-visual taxa.

Direct estimates of metabolic rate (oxygen consumption) have been augmented by measurements of the rates of key enzymes of intermediary metabolism which are useful proxies for metabolic potential in animals that are too fragile to survive trawling or retrieval to the surface (Sullivan and Somero, 1980; Dalhoff, 2004). These enzyme activities have correlated well with metabolic rates (Childress and Somero, 1979; Hochachka and Somero, 2002; Dalhoff, 2004). There is only one published study of the muscle enzyme activities of a hagfish (Davison et al., 1990). Many studies of demersal fishes have examined the enzymes citrate synthase (CS), pyruvate kinase (PK), malate dehydrogenase (MDH), and lactate dehydrogenase (LDH; Sullivan and Somero, 1980; Drazen and Seibel, 2007). CS is a key regulatory enzyme in the Krebs cycle and correlates with tissue mitochondrial density and aerobic capacity (Burness et al., 1999; Hochachka and Somero, 2002). MDH also regulates the Krebs cycle and may be involved in redox balance and other roles in intermediary metabolism (Siebenaller et al., 1982). PK and LDH are active in glycolysis with activities being an index of a tissue's anaerobic capacity (Childress and Somero, 1979). Scaling studies have additionally suggested, based on a general increase in LDH with body size (Somero and Childress, 1980; Childress and Somero, 1990; Somero and Childress, 1990), that LDH could be an indicator of burst locomotory capacity; increased glycolytic capacity allowing the fish to produce more power per gram muscle during burst swimming.

Our goal was to assess the metabolism of hagfishes. To accomplish this goal, we examined the metabolic rates, muscle enzyme activities, and proximate compositions (as proxy for locomotory capacity) of the two abundant hagfishes occupying different depth strata in the eastern North Pacific Ocean. An attractive characteristic of at least some hagfishes to metabolic studies is that they survive capture from depth. *Eptatretus stoutii* has been captured as deep as 1000 m but is only abundant between depths of about 20 and 400 m (Miller and Lea, 1972). *E. deani* can occur from 100 to 2700 m but is mostly found between 700 and 1200 m (Miller and Lea, 1972; Lauth, 1997).

2. Methods

Laboratory measurements of metabolic rate were made on animals obtained from trawling and trapping. Individuals of *E. deani* were caught between 500 and 1000 m in Monterey Bay, CA in October 2009 while individuals of *E. stoutii* were caught between 50 and 100 m off of San Diego, in November 2009. Despite its depth of capture, *E. deani* was alive and active at the surface and lived in captivity for months. An in situ measurement for *E. deani* was also obtained using the free-vehicle respirometer described in Drazen and Yeh (submitted for publication) deployed in Monterey Bay, California in October 2009. The respirometer consisted of four 100 L acrylic chambers each baited and equipped with a sealing door, circulation pump (SBE 5T), and oxygen optode (Aandera 4330) to allow for capture of fish and subsequent measurement of change in O_2 concentration ($[O_2]$, μmol L^{-1}) over time on the seafloor at ambient pressure and temperature. A control chamber was used on each deployment which closed 1 h after the system landed on the seafloor. The animal was recovered from the seafloor in the chamber and the end of the deployment.

Laboratory animals were held in a refrigerated room at a constant temperature of 5 °C in the dark. This temperature was chosen because it was within the range that the species are found and it was similar to the capture temperature of both species (4 °C at 1000 m depth to 9 °C at 100 m depth). All animals were acclimated for 48 h (two *E. deani*) to several weeks prior to experimentation. Food was withheld for a period of 4 days prior to experimentation, except for two *E. deani*, but the lack of fecal production in these animals suggests that specific dynamic action had little effect on the data. A 25.4×40.5×17.8 cm polyvinylchloride (PVC) chamber equipped with a pump (SBE 3T) for circulation and an oxygen optode (Aandera 4330) was fabricated for the measurement of O_2 consumption. All experiments were performed in the dark to minimize possible disturbance to the animals. Prior to starting each experiment, a control was performed for 2–4 h to evaluate any changes in $[O_2]$ over time in the absence of animals. Animals were then introduced to the chambers as carefully as possible. If slime was exuded the hagfish was returned to its holding tank, the respirometry chamber was cleaned, and the animal was introduced again later. Each animal was allowed to deplete the oxygen in the chamber to near zero over 40 to 70 h. Metabolic rates (μmol $g^{-1} h^{-1}$) for both in situ and laboratory measurements were derived by examining linear portions of the change in $[O_2]$ over time (h) then standardizing for mass (g). Although the animals were calm, the first ~4 h immediately after introduction were omitted to help ensure there was no effect of activity on the measured rates. The last portion of each experiment, when oxygen levels were below ~50 μmol L^{-1}, was also omitted as these levels were determined to be limiting. All hagfish were weighed within a day after the respiration measurement ended.

The effect of oxygen concentration on oxygen consumption was evaluated by plotting specific respiration against oxygen concentration. The critical oxygen pressure (P_c) below which the rate of oxygen consumption declined was estimated as in Seibel et al. (1997). Two regressions were performed, one for the portion of the relationship where the rate of oxygen consumption was maintained and another for the portion of the relationship where a steep decline in oxygen consumption occurred with decreasing oxygen concentration. The oxygen tension (kPa) at the intersection of these lines was used as the P_c.

We made comparisons of our data to that in the literature for other shallow living hagfishes. This approach required that the measurements were standardized to 5 °C as used in this study. To perform the scaling we used Q_{10} relationships available from the original references or assumed a Q_{10} of 2.3 which is an average for many fish species (Clarke and Johnston, 1999; Clarke, 2004). Data were available for 13 *E. stoutii* collected at 70 m depth off Oregon (Munz and Morris, 1965), three *E. hexatrema* from 10 m depth off South Africa (Kench, 1989), 8 *E. cirrhatus* (data given in the original reference as a single pooled average value) from depths <15 m off New Zealand (Forster, 1990), 10 *Myxine glutinosa* (again data pooled to one value) from a fjord in Sweden (Steffensen et al., 1984), 6 more *M. glutinosa* collected from ~100 m depth in the Gulf of Maine (Lesser et al., 1997), and a single value for *E. deani* measured in situ at 1230 m depth off southern California (Smith and Hessler, 1974).

The dorsal body musculature of hagfishes captured in trawls was sampled. Unlike teleosts, hagfish red and white muscle fibers are present in each muscle segment such that 70% of the volume of the muscle is white fiber and 30% is red/intermediate fiber in *M. glutinosa* (Flood, 1998). All muscle was frozen in liquid nitrogen and stored in a freezer at −80 °C until analysis.

Frozen muscle tissue was weighed and homogenized in nine volumes of 10 mM Tris HCl buffer (pH 7.55 at 10 °C) on ice in a Kontes Duall ground-glass homogenizer. Homogenates were centrifuged (5000 *g*) for 5 min after the completion of the CS assay and the resulting supernatant was used for all other assays. Supernatants were kept on ice, without further purification, until its use on the same day.

All assays were run in a total volume of 2.0 mL at 10 °C using a Shimadzu UV 1601 spectrophotometer with a temperature controlled water bath and water-jacketed 12-cell cuvette holder. Enzymatic activity is proportional to the change in absorbance at 340 nm (for

MDH, PK, and LDH) and at 412 nm (for CS) over time and is reported in international units (IU; μmol substrate converted to product per min) per gram tissue wet mass. Enzyme assays were run under saturating substrate conditions as follows. Citrate synthase (CS; EC 2.3.3.1): 0.1 mM dithiobis-nitrobenzoic acid, 0.1 mM acetyl CoA, 2 mM $MgCl_2$, 50 mM imidazole-HCl (pH 8 at 10 °C). Reaction was initiated by 0.5 mM oxaloacetate. Pyruvate kinase (PK, EC 2.7.1.40): 0.1 mM fructose 1,6 bisphospate, 5.0 mM ADP, 0.15 mM NADH, 10 U of LDH, 10 mM $MgSO_4$, 100 mM KCl, 80 mM Tris HCl (pH 7.8 at 10 °C). Reaction was initiated by 1.0 mM phospho(*enol*)pyruvate. Malate dehydrogenase (MDH; EC 1.1.1.37): 0.15 mM NADH, 0.5 mM oxaloacetate, 20 mM $MgCl_2$ 100 mM Tris HCl (pH 8.1 at 10 °C). Lactate dehydrogenase (LDH; EC 1.1.1.27): 0.15 mM NADH, 2 mM sodium pyruvate, 100 mM KCl, 80 mM imidazole-HCl (pH 7.8 at 10 °C).

A proximate analysis of the tissues was performed. Protein and lipid contents of dorsal musculature were determined in duplicate on tissue homogenized in distilled water. The bicinchoninic acid (BCA) protein assay (Smith et al., 1985) and lipid charring method (Marsh and Weinstein, 1966, as modified by; Drazen, 2002) used bovine serum albumin and glyceryl trioleate as standards, respectively. Tissue water content was determined by the difference between wet and dry masses of tissue portions following 24 h in a 60 °C drying oven, in triplicate.

The relationships between oxygen consumption or enzyme activity and body mass were examined using linear and power regressions. Interspecies comparisons in variables were made using nonparametric Mann–Whitney *U* tests, which are preferred in situations with small sample sizes and non-normal distributions. ANCOVA was used to compare variables between species if significant mass scaling was identified. All statistical tests were performed using Statistica 7.1 (http://www.statsoft.com).

3. Results

The oxygen consumption of 6 *E. deani* and 6 *E. stoutii* were measured in the laboratory at 5 °C (Table 1). One measurement of *E. deani* was also obtained in situ at a depth of 1010 m. At this depth the temperature was 4.0 °C and the ambient oxygen concentration was 19 μM. The oxygen concentration in the respirometry chamber declined to 15 μM; however the rate of oxygen consumption was measured only for concentrations well above the species critical oxygen concentration (see below). The in situ measurement was not different compared to those from the laboratory. There was no significant relationship between body mass and oxygen consumption of *E. stoutii* (Fig. 1). The relationship was significant for *E. deani* (oxygen consumption = $3.39\ \text{mass}^{-0.516\pm0.193}$, $r^2 = 0.59$, $p<0.05$) but over a small mass range preventing confidence in the magnitude of the scaling exponent. *E. deani* had a significantly greater rate of oxygen consumption than *E. stoutii* taking into account the differences in mass scaling between the species (ANCOVA, $p<0.01$).

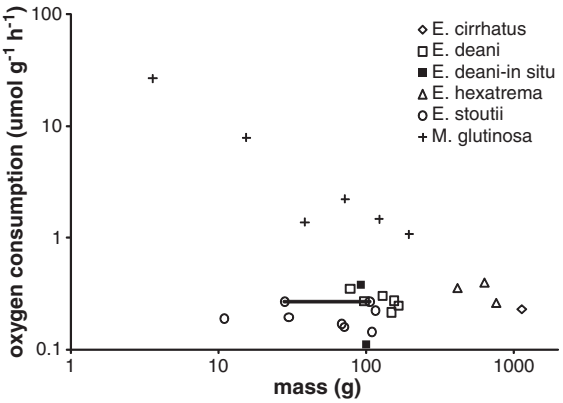

Fig. 1. Oxygen consumption as a function of body mass of the hagfish from this study and from the literature. The line between points for *E. stoutii* represents a mean for data on 13 individuals between 28 and 105 g that exhibited no significant body mass effects (Munz and Morris, 1965).

The hagfishes were extremely tolerant of low oxygen levels. The rate of oxygen consumption did not decline for either species until concentrations reached very low levels (Fig. 2). The critical oxygen pressure (P_c) for *E. deani* was 0.83 ± 0.12 kPa (equivalent to 12.5 μM), somewhat lower than that for *E. stoutii* (1.43 ± 0.21 kPa, equivalent to 21.4 μM). Thus the in situ measurement for *E. deani*, despite very low oxygen levels, occurred above this species' P_c. The P_c of *E. deani* was significantly lower than that of *E. stoutii* ($p<0.05$).

The enzyme data did reveal differences between the species (Table 2). The relationship between body mass and enzyme activities was examined (Fig. 3). There were generally lower MDH and CS activities in larger *E. deani*, but the regressions between enzyme activity and body mass were not significant. There were no significant relationships between *E. stoutii* enzyme activities and body mass. MDH and LDH were both significantly lower in *E. deani* (Mann–Whitney *U* test, $p<0.05$). CS, indicative of aerobic metabolism, was not significantly different between the two species despite significant differences in oxygen consumption. PK did not differ between the two species either.

The hagfish muscle tissue proximate composition was determined (Table 2). Both species had similar protein and water contents. The proportion of lipid in *E. deani* was ~5 times higher than that in

Table 1
Oxygen consumption (μmol $g^{-1}\,h^{-1}$) of individual hagfish at 5 °C.

Species	Mass (g)	O_2 cons.
Eptatretus deani	78	0.349
Eptatretus deani	97	0.269
Eptatretus deani	129	0.301
Eptatretus deani	148	0.215
Eptatretus deani	154	0.273
Eptatretus deani	165	0.246
Eptatretus deani — in situ	92	0.382
Eptatretus stoutii	11	0.190
Eptatretus stoutii	30	0.194
Eptatretus stoutii	68	0.170
Eptatretus stoutii	71	0.159
Eptatretus stoutii	109	0.144
Eptatretus stoutii	116	0.223

Note that the average temperature for the in situ measurement at 1010 m depth was 4 °C and respiration at 5 °C has been estimated using a Q_{10} of 2.3 (see Methods).

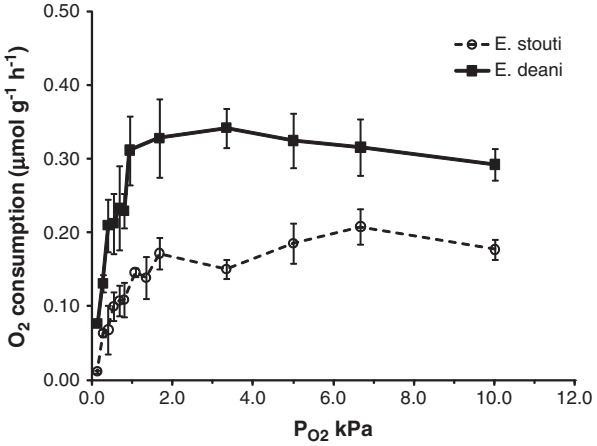

Fig. 2. Mean (±std. error) oxygen consumption of hagfishes as a function of oxygen concentration. P_c for *E. deani* was 0.83 ± 0.12 kPa significantly lower than that for *E. stouti*, 1.43 ± 0.21 kPa (see text).

Table 2
Mean (±std. dev.) of enzyme activities (units g^{-1}) and proximate composition (% of wet mass) of hagfish muscle.

Species	n	CS	MDH[a]	LDH[a]	PK	% water	% protein	% lipid[a]
Eptatretus deani	12/5	1.95 ± 0.34	29.6 ± 8.44	17.0 ± 5.56	11.4 ± 5.02	73.8 ± 1.44	9.7 ± 1.03	5.1 ± 2.70
Eptatretus stoutii	6/3	1.85 ± 0.60	50.1 ± 16.5	41.0 ± 20.7	17.7 ± 6.69	76.5 ± 5.09	11.0 ± 1.98	0.91 ± 0.27

Sample sizes (n) are given for enzyme activities then proximate composition. CS — citrate synthetase, MDH — malate dehydrogenase, LDH — lactate dehydrogenase, PK — pyruvate kinase.
[a] Indicates a significant difference between species.

E. stoutii (Mann–Whitney *U* test, $p<0.05$), and quite variable (range 2.4–8.6%).

4. Discussion

Our data corroborate earlier investigations of hagfishes which suggest that they have some of the lowest rates of metabolism of fishes. Our measurements for *E. stoutii* are similar to those in the literature (Munz and Morris, 1965) and this species has the lowest metabolic rate of the 5 species for which we could find data (but see measurement by Smith and Hessler, 1974 below). The deeper living *E. deani* has a higher rate of metabolism and our results (in situ and in the laboratory) give estimates 2.5–3 times higher than Smith and Hessler's (1974) in situ value. This difference prompts the question as to whether pressure influences the metabolic rate of this species. The lack of a difference between our in situ measurement and those made in the laboratory suggest otherwise. Data on other species of fishes lacking gasbladders (Meek and Childress, 1973; Belman and Gordon, 1979) and other animals (Childress, 1977; Thuesen and Childress, 1994) have come to similar conclusions. Low oxygen levels in situ are also not a likely explanation for the low rate measured by Smith and Hessler (1974) because oxygen concentration was 32 μmol L^{-1}, well above the P_c we measured for this species (Fig. 2). Although the reasons are not clear, the measurement taken by Smith and Hessler (1974) seems anomalously low.

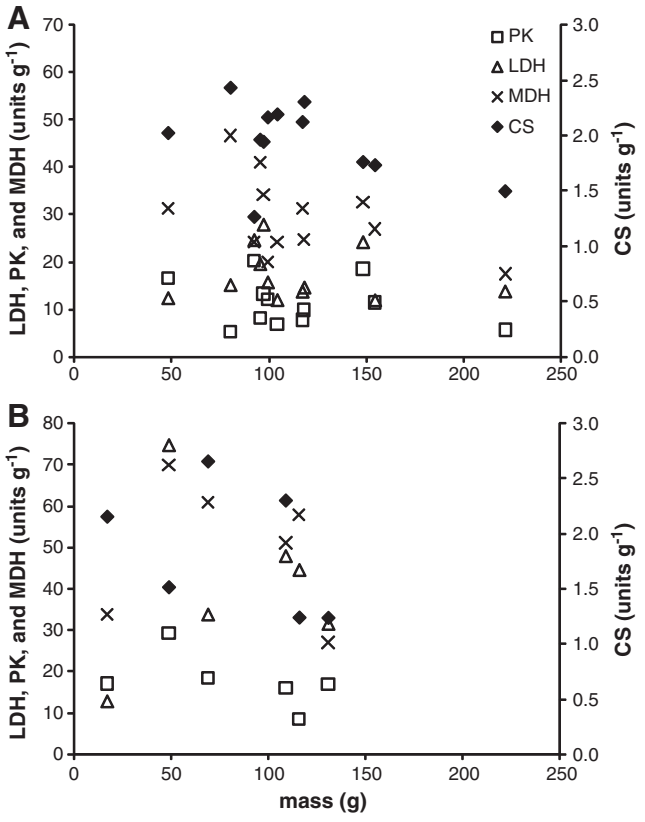

Fig. 3. Enzyme activities of A) *E. deani* and B) *E. stoutii* as a function of body mass. No significant correlations were found ($p>0.05$). CS — citrate synthetase, MDH — malate dehydrogenase, LDH — lactate dehydrogenase, and PK — pyruvate kinase.

The metabolic rates for *E. cirrhatus* (Forster, 1990) and *E. hexatrema* (Kench, 1989) are comparable to those of *E. deani*, but both of these species are much larger (Fig. 1). Our results for *E. deani* suggest a typical negative relationship between body mass and mass-specific metabolic rate. If similar scalings were evident in the larger *Eptatretus* spp. then their metabolic rates at similar sizes to *E. deani* and *E. stoutii* would be slightly higher. The data for *M. glutinosa*, the only studied *Myxine* species, suggest considerably higher metabolic rates than any of the *Eptatretus* spp. (Fig. 1, Lesser et al., 1997). Indeed at body mass of 50–100 g, the rates are comparable to those of shallow living and very active gadids such as *Boreogadus saidi* (Holeton, 1974) and *Gadus macrocephalus* (Paul et al., 1988). The study of Steffensen et al. (1984) provides metabolic rate estimates 2.5 times lower than those of Lesser et al. (1997), but still considerably higher than for the *Eptatretus* spp. The difference might be due to the stress of *M. glutinosa* in the chambers which has been suggested before (Forster, 1990). This species normally burrows in contrast to many eptatretids which can be found swimming or resting on the seafloor. Stress effects in Lesser et al.'s (1997) study may have been exacerbated by the short duration of the measurements (1–3 h) in contrast to the studies on *Eptatretus* spp. (10–72 h).

The ability of *E. stoutii* and *E. deani* to regulate their oxygen consumption at low oxygen concentrations was remarkable (Fig. 2). There is a pronounced oxygen minimum zone in the eastern Pacific (Levin, 2003). Along the California coast, oxygen concentrations decline to 23 μmol L^{-1} (~1.54 kPa or 0.5 mL L^{-1}) at ~500 m and rise above this level again at ~900 m (Levin, 2003). The habitat of *E. deani* encompasses this zone regularly and that of *E. stoutii* rarely (Miller and Lea, 1972; Lauth, 1997). As might be expected from their normal environmental oxygen concentrations (Seibel, 2011), the P_c of *E. deani* was somewhat lower than that of *E. stoutii* (0.83 vs. 1.43 kPa respectively, $p<0.05$). The P_c of *E. hexatrema* and *E. cirrhatus* which both inhabit shallow oxygen rich waters are 6.7 and 6.0 kPa, respectively (Kench, 1989; Forster, 1990). The heart of *M. glutinosa* is specifically adapted to low oxygen conditions, which it likely encounters in its burrows (Sidell and Beland, 1980; Baldwin et al., 1989), but no studies have evaluated this species' P_c. Our findings suggest that *E. stoutii* and *E. deani* will be very tolerant to recently documented expansions of oxygen minimum zones (Bograd et al., 2008; Stramma et al., 2008). In shallower shelf waters off California and Oregon there has been a recent increase in the frequency of low oxygen events which have caused high mortality in the benthic fauna (Chan et al., 2008). *E. stoutii* may be able to survive such events and potentially benefit from the carrion that is generated.

The enzyme activities of both species corroborated the oxygen consumption data, suggesting that hagfishes have very low metabolic rates (Table 2). Their activities were most similar to deep-sea benthic species (Drazen and Seibel, 2007) which is not surprising given hagfishes' rather sluggish behavior and poor burst swimming abilities (Davison et al., 1990). A study of the enzyme activities of *E. cirrhatus* found similar low activities of CS, PK, and MDH (Davison et al., 1990). The LDH activity was 4 fold higher than that of *E. stoutii* after accounting for differences in assay temperature. The *E. cirrhatus* were

considerably larger (~1000 g vs. 100 g) than the hagfish in this study which might explain the difference. LDH scales positively with fish size in teleosts, probably because the power required for acceleration increases rapidly with mass (Somero and Childress, 1980; Childress and Somero, 1990). However, neither *E. deani* nor *E. stoutii* exhibited size related scaling of LDH.

The visual interaction hypothesis makes several predictions about the metabolism of demersal fishes, a group which uses vision to mediate predator prey interactions. Benthopelagic species, which spend most of their time swimming over the seafloor, should have depth related declines in metabolic rate (after correcting for mass and temperature). Their locomotory abilities and hence metabolic rates decline with depth as the need to interact with predators and prey over long distances declines (as a function of light levels). There is very little data on deep-sea demersal fish metabolism (Drazen and Seibel, 2007; Drazen and Yeh, submitted for publication) but data show that benthopelagic macrourids have reduced metabolic rates compared to shallow living and phylogenetically related species. Benthic fishes which spend most of their time resting on the seafloor are not predicted to have depth related declines in metabolic rate after mass and temperature have been accounted for. In contrast to the water column, the benthic habitat affords both predators and prey the opportunity for crypsis (Hamner, 1995) and thus the potential for sedentary lifestyles at all depths. In support of this contention, benthic scorpaenid fishes show little difference between shallow and deep living species (Drazen and Seibel, 2007). The VIH also predicts no change in hagfish metabolic rate with depth because these animals are benthic (sometimes burrowing) and because they are functionally blind. The distances over which they interact with predators and prey should not change with increasing depth and declining light levels (Martini, 1998), so neither should their metabolic rates.

The analysis of hagfish respirometry suggests a more complicated situation than the VIH predicts. With or without the data for *M. glutinosa*, it is clear that significant variation exists in the metabolic rates of hagfishes even after accounting for temperature and body mass effects. However, this variation is not explained by habitat depth. *E. deani*, the deepest living species, has rates that are intermediate, rather than the lowest amongst the species. The species with the lowest metabolic rate, *E. stoutii*, lives somewhat deeper than *E. hexatrema* (most abundant between 10 and 50 m), at comparable depths to *E. cirrhatus* (30–300 m; Fernholm, 1998), and is normally found shallower than *E. deani*. The proximate chemistry of the muscle tissue of *E. stoutii* and *E. deani* (Table 2) was indicative of similar locomotory capacity because their protein and water contents were similar (Childress and Nygaard, 1973; Drazen, 2007) but no data are available for other species. For the enzyme activities, there were no differences in CS or PK between the *E. deani* and *E. stoutii* species (Table 2) as would be predicted by the VIH but MDH and LDH were significantly higher in *E. stoutii* contrary to expectations. It is difficult to explain elevated activities for one aerobic (MDH) and one anaerobic (LDH) enzyme, in particular, because these two enzymes were much more variable in *E. stoutii* (Table 2 and Fig. 3b). Perhaps this reflects a more heterogenous distribution of muscle fiber types in the dorsal muscle of this species. More data on the enzyme activities and respiration of hagfishes is needed to better interpret interspecific differences and definitively evaluate the predictions of the VIH.

The much greater lipid stores found in the deeper living *E. deani* (Table 2) may be the result of differences in food supply or feeding strategies. Hagfishes feed on benthic infauna such as worms and they also scavenge on animal carcasses that have sunken to the seafloor (Martini, 1998; Yeh and Drazen, 2011). Benthic biomass, a proxy for benthic food supply, decreases exponentially with depth (Gage and Tyler, 1991). It may be that deeper living species rely to a greater extent on sporadic but energy rich carrion falls. Species living in environments with low and sporadic food supply often have greater lipid stores (Bailey and Robison, 1986) and there is an increase in lipid content with depth across a diversity of benthic fishes (Drazen, 2007). This may be true for hagfishes too.

In summary, both our oxygen consumption and enzyme activity data suggest very low rates of metabolism in hagfishes similar to most previous studies. Both species can regulate their oxygen consumption in low oxygen environments, suggesting that they may not be adversely affected by expansion of oxygen minimum zones or temporary hypoxic events on the continental shelf. There are large differences in metabolism between hagfishes, particularly between *M. glutinosa* and *Eptatretus* spp., which are not explainable by body size, temperature, habitat depth, or the VIH. Additional data from a greater number of species across a wider depth range will contribute to our understanding of the energetics of this unique group of animals.

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