Mar Biol (2012) 159:1713–1731
DOI 10.1007/s00227-012-1960-3

ORIGINAL PAPER

Metabolic enzyme activities in shallow- and deep-water chondrichthyans: implications for metabolic and locomotor capacity

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Received: 5 November 2011 / Accepted: 8 May 2012 / Published online: 5 June 2012

Abstract Biochemical indices of white (WM) and red muscle (RM) aerobic and anaerobic metabolic capacity were measured in 14 species of benthic and benthopelagic chondrichthyans from a depth of ~90 to 2,200 m to evaluate the relationship between metabolic capacity and depth of occurrence, phylogeny, and locomotor mode. Maximal activities of the enzymes citrate synthase, malate dehydrogenase (MDH), lactate dehydrogenase (LDH), and pyruvate kinase (PK) were analyzed in muscle tissue at 10 °C. These were combined with previously published elasmobranch data in order to represent a comprehensive range of depths, phylogeny, and locomotor modes (i.e., benthic, benthopelagic, pelagic). Significant decreases in WM PK and LDH activities and a lack of significant trends in RM enzyme activities with increasing median depth of occurrence (MDO) indicate a depth-related reduction in both burst-locomotor and metabolic capacity. These trends are consistent with the "visual-interactions hypothesis." Phylogeny and locomotor mode had little influence on enzyme activities compared to MDO, and the present study suggests similar activities in co-occurring demersal sharks and rays. Overall, the present study indicates low metabolic capacities in deep-sea chondrichthyans, which is important to consider when managing deep-sea fisheries.

Communicated by K. D. Clements.

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Introduction

There is a need to study the ecological importance of understudied marine taxa, such as chondrichthyan fishes, across all depth ranges, in light of increasing fishing pressures and progressively deepening fisheries. Deep-sea fisheries appear to have already reached the maximum attainable depths known for chondrichthyan fishes (Priede et al. 2006; Garcia et al. 2008). Understanding the metabolism of elasmobranchs is important in terms of their overall ecological importance. Metabolism, the process of energy assimilation and consumption in individuals, strongly influences energetic processes such as growth, reproduction, locomotion, and resource utilization (Brown et al. 2004; Drazen and Seibel 2007). However, metabolism is challenging to quantify due to the difficulty of measuring important energetic requirements such as swimming speeds, activity patterns, and feeding rates of elasmobranchs (Dickson et al. 1993), especially deep-living species.

In place of direct measurements, maximal activities of muscle enzymes have been used as indirect estimates of tissue aerobic and anaerobic capacity (Sullivan and Somero 1980; Siebenaller et al. 1982; Torres and Somero 1988; Dickson et al. 1993; Thuesen and Childress 1993a, b; Vetter and Lynn 1997; Seibel et al. 2000; Bernal et al. 2003; Treberg et al. 2003; Dalhoff 2004; Drazen and Seibel 2007; Seibel and Drazen 2007; Ombres et al. 2011). The enzymes pyruvate kinase (PK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and citrate synthase (CS) are often assayed due to their key roles in adenosine triphosphate (ATP) production necessary for muscle contraction (Siebenaller et al. 1982; Childress and Somero 1979). Several studies have found that the activities of these enzymes correlate well with (and are useful proxies

for) metabolic rate (Childress and Somero 1979; Dalhoff 2004; Drazen and Seibel 2007). Several lines of evidence suggest that CS, a key enzyme of the TCA cycle, is a good indicator of aerobic capacity. Activities correlate with mitochondrial density in fishes including elasmobranches (Moyes et al. 1992; Duong et al. 2006) and scale positively with whole animal metabolic rate (Alp et al. 1976; Torres and Somero 1988). Similar to aerobic metabolic rate, CS negatively scales with increasing body size (Somero and Childress 1980). MDH is another enzyme of the TCA cycle and is also involved in shuttling reducing equivalents between the mitochondrion and the cytoplasm. It has additionally been used as an indicator of aerobic metabolism (Sullivan and Somero 1980; Siebenaller et al. 1982; Ombres et al. 2011). PK catalyzes the last reaction in glycolysis leading to pyruvate formation. Although pyruvate can be moved to the mitochondria for oxidation via the TCA cycle, it can also be used in anaerobic glycolysis. Arguably, the high flux requirements of anaerobic glycolysis relative to aerobic metabolism make PK values a good indicator of overall anaerobic metabolic capacity (Somero and Childress 1980), and past studies have found a positive interspecific correlation between PK and LDH (Childress and Somero 1979; Sullivan and Somero 1980; Ombres et al. 2011). LDH catalyzes the final reaction of anaerobic glycolysis, ultimately producing lactate and maintaining redox balance to allow ATP production to continue anaerobically. As a result, activity values are indicative of anaerobic capacity and burst-locomotor capacity in white muscle, which powers burst swimming, interspecifically (Childress and Somero 1979; Somero and Childress 1980; Dalhoff 2004), although not intraspecifically in some species (Gibb and Dickson 2002).

Measurements of metabolic rates and metabolic enzyme activities of deep-dwelling chondrichthyans are almost entirely lacking, while numerous data on the differences between deep and shallow-living species of teleosts are available. Teleost studies have shown a general decrease in metabolic enzyme activities of white muscle with minimum depth of occurrence (Childress and Somero 1979; Sullivan and Somero 1980; Drazen and Seibel 2007). Thus, it may be reasonable to assume similar depth-related trends in chondrichthyan fishes. Previous studies on muscle tissue enzymatic activities of chondrichthyan species have focused on shallow-living, active, pelagic species (Crabtree and Newsholme 1972; Alp et al. 1976; Zammit et al. 1978; Sullivan and Somero 1980; Moon and Mommsen 1987; Dickson et al. 1988, 1993; Bernal et al. 2003). This leaves a major gap in the knowledge of the metabolism of deep-living chondrichthyan fishes. Treberg et al. (2003) is the only study, thus far, to make a direct comparison between species of shallow and deep-living elasmobranchs. Their results mimic trends seen in teleost studies, with the deep-living squaloid shark, *Centroscyllium fabricii*, having substantially lower activities of the enzymes PK and LDH in fast-glycolytic or white muscle (WM), and generally lower enzyme activities in slow-oxidative or red muscle (RM) relative to a shallow-living relative, *Squalus acanthias*.

The leading hypothesis for this decline in metabolic rate and metabolic enzyme activities with depth is the "visual-interactions hypothesis" (VIH). The VIH, based principally on data for pelagic species, suggests that declining light levels with depth decreases the selective pressure for high locomotor capacity in species with visual capabilities, because the reactive distances between predator and prey are reduced. Metabolism subsequently declines due to the cost of maintenance and operation associated with high-performance locomotion (Childress et al. 1990a; Somero 1992; Childress 1995; Drazen and Seibel 2007). This theory is supported by evidence that non-visual animals, such as chaetognaths, medusa, and worms, do not exhibit depth-related declines in metabolic rate, but visual taxa, such as cephalopods, crustaceans, and teleosts, do (Sullivan and Somero 1980; Thuesen and Childress 1993a, b; Seibel et al. 1997; Seibel and Drazen 2007). Among visual taxa, metabolic rates have been shown to decline drastically in the first 500 m, and level off below approximately 1,000 m where visible light is absent (Childress 1995; Warrant and Locket 2004). Although it is generally recognized that high hydrostatic pressure does not result in lower metabolic rates (Seibel and Drazen 2007), food availability is often given as an additional potential factor for decreases in metabolic rate with depth (Childress 1971; Smith and Hessler 1974; Dalhoff 2004) due to the existence of an order of magnitude exponential decline in animal biomass with depth (Haedrich and Rowe 1977; Thurston et al. 1994; Priede et al. 2006). However, whether food availability is an important determinant of metabolic rate is highly debated (Ikeda 2008).

Previous studies on teleosts suggest that interspecific differences in feeding and locomotor modes measured in pelagic and benthic species may partially explain differences in WM enzyme activity (Sullivan and Somero 1980; Siebenaller et al. 1982; Dickson et al. 1993). During short-duration burst-swimming activity (e.g., predator avoidance and prey capture) powered by WM, ATP is produced by anaerobic glycolysis (involving LDH and PK) through the degradation of glycogen stores to lactate, and aerobic pathways of ATP production are of little importance (Bilinski 1974; Sullivan and Somero 1980). This reveals the potential for wide variation in glycolytic enzyme activities with different burst-locomotor capacities. Skates, thought to be ambush predators that are less active than other chondrichthyan species, and rays have lower metabolic enzyme activities in muscle tissue compared with both

active pelagic and demersal sharks (Dickson et al. 1993; Carlson et al. 2004). More data on the enzyme activities of slow-oxidative muscle (or RM), which powers sustained slow swimming (Bone 1966; Somero and Childress 1980), could greatly expand our understanding of chondrichthyan metabolism and locomotion, because almost all muscle enzyme activity data from chondrichthyans are from WM.

Protein and water content have been used as additional proxies for locomotor capacity. Pelagic fish and crustaceans show a decline in protein and a rise in water contents, along with a decrease in body robustness and potential locomotor capacity, with depth (Childress and Nygaard 1973, 1974; Bailey and Robison 1986; Stickney and Torres 1989; Childress et al. 1990b). Similar correlations may be found between locomotor modes and protein and water contents in chondrichthyans.

The present study evaluated the largest compiled data set of chondrichthyan metabolic enzyme activity in both white and red muscle. The focus of the present study was to evaluate the influence of depth on metabolic enzyme activities and tissue composition (lipid, water, and protein contents) of benthic and benthopelagic chondrichthyans as proxies for metabolic and locomotor capacity and to test the predictions of the VIH. In addition, we used the enzymatic data to explore differences with regard to phylogeny and general locomotor mode, in order to learn more about the ecology and biology of species that are difficult to observe in the natural environment.

Materials and methods

Tissue collection

Elasmobranchs were caught utilizing along-contour trawls during two cruises in Monterey Bay, California, in April and October of 2009 at target depths of 100–2,000 m. Additional specimens were caught in June 2010 during a Fishery Resource Analysis and Monitoring (FRAM) Division (NOAA/NMFS) groundfish slope survey sampling from Coos Bay, Oregon, to San Francisco, California.

Specimens were placed on ice immediately upon being sorted from the trawl and kept on ice for up to 1 h before sampling. All specimens were responsive when sorted from the trawl. WM tissue was excised from sharks and chimaeras dorsolaterally below the first dorsal fin, and dorsally from the thickest part of the pectoral fin and next to the vertebrae in skates. Most skates are known to be undulatory appendage propulsors that swim by passing waves down the pectoral fin in the posterior direction (Webb 1993). Therefore, both pectoral fin and vertebral muscle in skates were sampled for within-individual comparisons. Sampling *Torpedo californica* presented difficulty because both specimens were small juvenile males (0.49–0.70 kg). The pectoral fins of this species are composed mainly of the electric organ, and extra care was taken to sample only locomotor WM tissue.

RM tissue was also sampled for some species. RM was sampled from only one shark species in the present study, the two largest specimens of *Somniosus pacificus*. RM samples were obtained in sufficient amounts dorsally above the WM sample in all skates studied except *Raja inornata*. Tissue samples were placed in cryovials and frozen in liquid nitrogen until being transferred to a -80 °C freezer. Samples were stored for up to 22 months prior to analysis. Dickson et al. (1993) showed that storage for up to 44 months had no affect on the activities of the enzymes CS, PK, and LDH in elasmobranchs.

Enzyme assays

The maximal activities of the enzymes CS, MDH, LDH, and PK were measured. Frozen muscle tissue was weighed and homogenized on ice in nine volumes of ice-cold 10 mM Tris–HCl buffer (pH 7.55 at 10 °C) in a Kontes Duall ground-glass homogenizer attached to a motorized system to ensure full homogenization. CS was assayed using unspun homogenates. Homogenates were then centrifuged (5,000*g*) for 5 min upon completion of the CS assay. Care was taken to avoid any resulting lipid layer, and the supernatant was used for LDH, PK, and MDH assays. Supernatants were kept on ice, without further purification, until they were used 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 water-jacketed 12-cell cuvette holder connected to a temperature-controlled water bath. Assays were based on protocols on fish muscle enzymes (Childress and Somero 1979; Treberg et al. 2003). A temperature of 10 °C was chosen because it is within the temperature range experienced by most of the species studied. Enzymatic activity is proportional to the change in absorbance at 340 nm (for MDH, PK, and LDH) and 412 nm (for CS) over time and is reported in international units (U; μmol substrate converted to product per min) per gram tissue wet mass. Enzyme assays were run under saturating substrate conditions, as determined by preliminary assays run with various dilutions of the homogenate with homogenization buffer. The final conditions for each assay were as follows: citrate synthase: 0.1 mM dithiobis-nitrobenzoic acid, 0.1 mM acetyl CoA, 2 mM $MgCl_2$, 50 mM imidazole HCl (pH 8 at 10 °C). Absorbance changes were adjusted for control rates determined in the presence of enzyme prior to the addition of 0.5 mM oxaloacetate, which initiated the reaction. Pyruvate kinase: 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). The PK reaction was initiated by the addition of 1.0 mM phospho(enol) pyruvate. Malate dehydrogenase: 0.15 mM NADH, 0.5 mM oxaloacetate, 20 mM $MgCl_2$ 100 mM Tris–HCl (pH 8.1 at 10 °C). Lactate dehydrogenase: 0.15 mM NADH, 2 mM sodium pyruvate, 100 mM KCl, 80 mM imidazole HCl (pH 7.8 at 10 °C). Reactions for MDH and LDH were initiated by the addition of supernatant.

Enzyme activities have been shown to scale with body size—with a general increase in the activity of glycolytic enzymes LDH and PK and a decrease in the activity of CS with increasing body mass (Somero and Childress 1980). Regression analyses were performed between body mass and individual enzyme activities for both RM and WM for species with sample sizes large enough for analysis.

Previous studies have run all assays, including CS, after centrifuging homogenates, but different studies have used different centrifugation speeds and durations (see Somero and Childress 1980; Sullivan and Somero 1980; Dickson et al. 1993; Bernal et al. 2003; and Treberg et al. 2003, for example). The clear (particle-free) supernatant is assayed in order to reduce the background noise in the spectrophotometer. CS is found within the mitochondrion (Childress and Somero 1979), and significant activity may be lost in the assay due to the process of spinning out mitochondria rich particles in the homogenate. In order to adjust for differences in protocols, and to make a more accurate comparison between this and previous studies, the effects of centrifugation on CS activity were analyzed across a range of species for both teleosts (*Microstomus pacificus*, *Anoplopoma fimbria*, and *Coryphaenoides acrolepis*) and elasmobranchs (*Raja inornata*, *Apristurus brunneus*, and *Amblyraja badia*) using unspun homogenates, quick spun supernatants (20 s at 5,000*g*), and full spun supernatants (5 min at 5,000*g*). Comparisons of the present studies data with that from the literature for other chondrichthyan species of varying depth ranges and activity levels were then made. Previous data on elasmobranchs centrifuged their samples at speeds ranging from 5,000 to 12,000*g* for 5–10 min (Moon and Mommsen 1987; Dickson et al. 1993, 1988; Bernal et al. 2003; Treberg et al. 2003). For the purposes of comparison, these previous data are considered full spun.

Median depth of occurrence

Previous studies on teleosts and other animals have examined depth-related trends in metabolic variables using the minimum depth of occurrence for each species (for example Childress and Somero 1979; Sullivan and Somero 1980; Drazen and Seibel 2007). The minimum depth of occurrence is defined as the depth below which 90 % of the adult population of a given species is captured (Childress 1995; Seibel and Drazen 2007). This approach takes into account the fact that a given fish may not always occupy a single depth due to possible diel and ontogenetic migration (Jacobson et al. 2001; Collins et al. 2005). However, many species of elasmobranch are known to have seasonal or ontogenetic depth shifts and to segregate in depth and location by sex, age of maturity, and/or size (Ebert 2003). In this case, the minimum depth of occurrence may be representative of only a small proportion of the population. We used the median depth of occurrence (MDO) in order to be more representative of the range of habitat depths occupied. Minimum and maximum depths of occurrence were obtained for all species from the literature, and the median was taken between the two values (Table 1; Ebert 2003; Kyne and Simpfendorfer 2010; Compagno et al. 2005; Froese and Pauly 2004).

Tissue composition

Protein, lipid, and water contents in WM were quantified to look for potential trends with depth. Protein and lipid content of dorsal WM tissue were measured in duplicate on tissue homogenized in distilled water. The bicinchoninic acid (BCA) protein assay (Smith et al. 1985) was used with bovine serum albumin as a standard. Lipids were extracted according to Bligh and Dyer (1959) as modified by Reisenbichler and Bailey (1991) and assayed using the sulfuric acid charring method of Marsh and Weinstein (1966) using glyceryl trioleate as a standard. Tissue samples ($\sim$0.05–0.2 g) were also dried in a 60 °C oven in triplicate for 24 h, or until dry, to measure water content by taking the difference between wet and dry mass of the tissue.

Statistical analyses

A Q_{10} of 2.0 was used to adjust the literature data to a temperature of 10 °C for comparisons with the present data where needed. Model I and II regression analyses were applied to explore the relationships between depth, body mass, proximate chemistry for tissue composition, and enzymatic activity. A model II regression (Ricker 1973; Laws 1997) was used when comparing enzyme activities to median depths of occurrence because both variables are not controlled, and hence, both contain error. A log–log transformation was conducted prior to model II regressions when necessary, as model II regressions can only be completed with linear regressions. Data from the previously published literature were included in the analysis of trends in enzyme activity with depth and in the analysis of the potential effects of both phylogeny and locomotor mode on WM enzyme activity (see Table 1 for adjusted

Table 1 Summary of the mean enzyme activities (U g^{-1} wet mass) of white muscle (WM), both pectoral (WMp) and vertebral (WMv), and red muscle (RM) at 10 °C (means ±1 standard deviation where applicable)

Species	*n*	Fish wt range (kg)	Min depth occurrence (m)	Max depth occurrence (m)	MDO (m)		TCA metabolism		Glycolytic metabolism		Source
							CS	MDH	PK	LDH	
Sharks											
Alopias vulpinus (common thresher shark)	6	6.3–43.1	0	366	183	WM	1.03 ± 0.32			207.4 ± 41.15	[2]
						RM	17.67 ± 2.01			100.7 ± 10.55	
Apristurus brunneus (brown catshark)	7	0.048–1.0	33	1,298	665.5	WM	0.60 ± 0.22	7.27 ± 1.54	14.56 ± 7.63 (6)	17.55 ± 5.94	This study
Carcharhinus acronotus (Atlantic blacknose shark)	2	0.5–3.1	9	64	36.5	WM				532.45	[2]
Carcharhinus limbatus (blacktip shark)	1		0	64	32	WM	0.78		63.8	385	[4]
						RM	30.63		26.6	46.85	
Carcharhinus plumbeus (sandbar shark)	2		1	280	140.5	WM	0.96		80	193	[4]
						RM	12.19		25.6	58.1	
Centroscyllium fabricii (black dogfish)	5	0.03–0.09	180	1,600	890	WM	0.84 ± 0.48	5.73 ± 1.46	26.02 ± 7.42	50.77 ± 13.79	[8]
						RM	11.05 ± 1.83	106.21 ± 13.72	37.34 ± 12.78	47.38 ± 11.02	
Isurus oxyrinchus (shortfin mako shark)	37	4.8–60.8	0	500	250	WM	1.57 ± 0.33		196.8 ± 37.8	551.35 ± 27.0	[2, 5]
						RM	15.19 ± 1.46		59.9 ± 18.8	112.78 ± 9.6	
Lamna ditropis (Salmon shark)	2	127–136	0	375	187.5	WM	1.9			627.25	[2]
						RM	23.02			124.95	
Mustelus californicus (gray smoothhound shark)	2	0.19–0.26	0	46	23	WM	0.83		126.65	339.70	[5]
Parmaturus xaniurus (filetail catshark)	4	0.18–0.33	91	1,251	671	WM	0.73 ± 0.11	9.38 ± 4.15	46.68 ± 3.66	12.98 ± 9.92	This study
Prionace glauca (blue shark)	4	34–59	1	350	175.5	WM	0.74 ± 0.27		51.65 ± 2.75	74.55 ± 9.55	[5]
						RM	14.51 ± 4.26		14.35 ± 1.45	35.7 ± 3.95	
Rhizoprionodon terraenovae (Atlantic sharpnose shark)	8	0.1–0.7	10	280	145	WM				462.35 ± 26.15	[2]
Scyliorhinus canicula (dogfish)	3		10	780	395	WM	1.18		96.17	116.67	[1, 3, 9]
						RM	90.65		35.71	38.89	

Table 1 continued

Species	*n*	Fish wt range (kg)	Min depth occurrence (m)	Max depth occurrence (m)	MDO (m)		TCA metabolism		Glycolytic metabolism		Source
							CS	MDH	PK	LDH	
Somniosus pacificus (Pacific sleeper shark)	3	2.84–49.18	0	2,000	1,000	WM	0.49 ± 0.08	7.29 ± 2.50	43.44 ± 6.63	37.28 ± 13.10	This study
						RM	6.80 ± 1.54 (2)	103.02 ± 57.85 (2)	22.23 ± 5.30 (2)	56.02 ± 35.57 (2)	
Sphyrna lewini (scalloped hammerhead shark)	10	0.5–0.8	0	512	256	WM	0.76 ± 0.23			323.3 ± 19.85	[2]
						RM	22.05 ± 0.91			76.45 ± 6.2	
Squalus acanthias (spiny dogfish)	7		0	1,460	730	WM	0.98	4.78 ± 0.54	42.01	116.39	[7, 8]
						RM	18.49 ± 3.1	72.12 ± 8.41	41.86 ± 7.44	28.43 ± 7.04	
Triakis semifasciata (leopard shark)	9	0.14–0.81	1	91	46	WM	0.72 ± 0.25		117.55 ± 12.30	330.60 ± 10.75	[5]
						RM	13.80 ± 1.43		28.90 ± 2.30	94.70 ± 4.10	
Rays											
Amblyraja badia (broad skate)	6	0.04–2.94	846	2,324	1,585	WMv	0.98 ± 0.24 (3)*	12.90 ± 2.15 (3)	35.72 ± 9.30 (3)	54.29 ± 16.55 (3)	This study
						WMp	2.65 ± 0.66	20.46 ± 5.61	34.77 ± 9.41	48.07 ± 12.60	
						RM	15.35 ± 7.11 (5)	99.51 ± 25.68 (5)	42.08 ± 8.78 (5)	50.22 ± 11.78 (5)	
Bathyraja abyssicola (deep-sea skate)	4	3.87–10.02	362	2,906	1,634	WMv	0.37 ± 0.05 (3)	14.13 ± 7.57 (3)	28.44 ± 6.90 (3)	64.57 ± 21.35 (3)	This study
						WMp	0.70 ± 0.13	14.42 ± 3.59	32.00 ± 10.25	46.32 ± 15.44	
						RM	22.95 ± 1.45 (3)	137.87 ± 19.64 (3)	35.28 ± 0.70 (3)	55.46 ± 54.47 (3)	
Bathyraja kincaidii (sandpaper skate)	8	0.12–1.04	55	1,372	713.5	WMp	1.11 ± 0.78 (6)	15.73 ± 4.67 (6)	54.71 ± 16.89 (6)	70.30 ± 15.72 (6)	This study
						RM	14.61 (1)	133.72 (1)	33.62 (1)	87.0 (1)	
Bathyraja microtrachys (fine-spined skate)	5	1.18–1.97	1,995	2,900	2,447.5	WMv	0.41 ± 0.05 (3)*	10.05 ± 1.51 (3)	26.10 ± 11.57 (3)	45.26 ± 17.60 (3)	This study
						WMp	1.68 ± 1.01	21.89 ± 8.64 150.51 ± 8.14 (4)	41.59 ± 21.84	59.93 ± 27.92	
						RM	23.61 ± 3.65 (4)		40.17 ± 11.53 (4)	82.84 ± 11.26 (4)	
Bathyraja trachura (roughtail skate)	7	1.46–3.9	213	2,550	1,381.5	WMv	0.41 ± 0.06 (3)	14.64 ± 3.58 (3)	57.80 ± 14.82 (3)	66.63 ± 14.55 (3)	This study
						WMp	0.57 ± 0.16	15.64 ± 4.45	64.85 ± 14.79	63.67 ± 14.20	
						RM	17.42 ± 3.07 (5)	140.93 ± 17.76 (5)	55.17 ± 14.75 (5)	112.79 ± 10.51 (5)	
Platyrhinoidis triseriata (thornback ray)	4	0.09–0.19	1	137	69	WM	0.66 ± 0.26		106.60 ± 23.70	185.15 ± 14.20	[5]
Raja binoculata (big skate)	6	0.82–14.85	3	800	401.5	WMp	1.70 ± 0.19	20.26 ± 6.89	67.98 ± 19.48	85.63 ± 21.38	This study
						RM	29.41 ± 2.97 (3)	186.04 ± 16.13 (3)	105.71 ± 12.97	149.66 ± 14.42 (3)	
Raja erinacea (little skate)	6	164.5	1	329	165	WM	1.22 ± 0.68	11.96 ± 1.07	49.77 ± 3.1	104.9 ± 7.81	[6]
						RM	20.93 ± 4.07	94.67 ± 11.9	52.03 ± 12.57	59.34 ± 4.16	
Raja inornata (California skate	5	0.32–1.4	17	671	344	WMp	1.35 ± 0.17 (4)	17.85 ± 3.20 (4)	59.87 ± 16.19 (4)	106.79 ± 8.60 (4)	This study

Table 1 continued

Species	*n*	Fish wt range (kg)	Min depth occurrence (m)	Max depth occurrence (m)	MDO (m)		TCA metabolism		Glycolytic metabolism		Source
							CS	MDH	PK	LDH	
Raja rhina (longnose skate)	17	0.06–6.12	9	1,069	539	WMv	0.74 ± 0.15	18.11 ± 5.18 (3)	61.69 ± 32.18 (3)	67.32 ± 24.38 (3)	This study
						WMp	1.15 ± 0.35	15.30 ± 4.84	33.05 ± 12.46 (13)	51.23 ± 18.03	
						RM	21.98 ± 7.01 (2)	156.94 ± 36.35 (2)	62.01 ± 12.75 (2)	83.55 ± 22.89 (2)	
Raja stellulata (Pacific starry skate)	1	1.48	18	732	375	WMp	0.74	18.72	52.94	80.07	This study
						RM	19.55	154.63	65.51	92.35	
Rhinobatos productus (shovelnose guitarfish)	8	0.06–1.00	1	91	46	WM	0.78 ± 0.28		145.70 ± 16.0	287.95 ± 55.85	[5]
Torpedo californica (Pacific torpedo ray)	2	0.49–0.70	3	200	101.5	WMv	1.29 ± 0.07	9.88 ± 1.38	39.93 ± 0.75*	50.10 ± 1.16*	This study
						WMp	1.05	6.62	20.25	15.51	
Urobatis halleri (round sting ray)	3	0.09–0.31	1	91	46	WMv	0.62 ± 0.21		91.40 ± 7.65	184.40 ± 20.50	[5]
Chimaera											
Hydrolagus colliei (spotted ratfish)	8	0.09–1.13	1	971	486	WM	1.54 ± 0.19	12.39 ± 1.91	36.04 ± 6.56 (7)	62.89 ± 18.18	This study

Literature data were adjusted for CS [CS = 1.29*(full spun CS) + 0.41] and for temperature using a Q_{10} of 2.0. Median depths of occurrences were obtained from Ebert (2003), Froese and Pauly (2004), Compagno et al. (2005), and Kyne and Simpfendorfer (2010). If the sample size was different than the reported "*n*" for a given assay, the number was reported in parentheses below the value. For ray species, differences in WMp and WMv were evaluated using paired *t* tests. Significant differences (p value < 0.05) are indicated with a *

Sources [1] Alp et al. 1976, [2] Bernal et al. 2003, [3] Crabtree and Newsholme 1972, [4] Dickson et al. 1988, [5] Dickson et al. 1993, [6] Moon and Mommsen 1987, [7] Sullivan and Somero 1980, [8] Treberg et al. 2003, [9] Zammit et al. 1978

literature values used). Differences between pectoral and vertebral WM enzyme activities of rays in the present study were assessed using a paired *t* test. A Kruskal–Wallis test with a Tukey–Kramer post hoc procedure was used to test for interspecific differences in enzymatic activity in the data and also for phylogenetic effects on WM enzyme activity in both the data and literature values (excluding groups with only one value). All tests were run at a significance level of $\alpha = 0.05$ using Statistica 7.1 (Stat Soft, Inc., Tulsa, Oklahoma).

A hierarchical cluster analysis was performed using CS and LDH values for all available data in order to further test for interspecific differences. MDH and PK were not included due to the paucity of data in the previous literature. The data were transformed into a Bray–Curtis similarity matrix, and cluster distances were determined by using group average values. This allows for comparisons that take into account enzymes involved in both aerobic and anaerobic metabolism. Prior to analysis, each variable was standardized to the maximum value from 0 to 100. Cluster analyses were performed with Primer 6.1 (PRIMER-E Ltd.).

Results

Fourteen elasmobranch species were sampled over a broad depth range in the present study: 9 skate species from ~90 to 570 m and 830 to 2,200 m; 3 shark species from ~450 to 1,200 m; 1 species of torpedo ray at ~90 m; and 1 species of chimaera from ~80 to 540 m (Table 1). Although evolutionarily divergent, the chimaera, *Hydrolagus colliei*, was included in the present study, as Chimaeriformes have been shown to have similar metabolic organization to elasmobranchs (Speers-Roesch et al. 2006). All chondrichthyans caught in the 39 trawls conducted from approximately 100 to 2,000 m in Monterey Bay were sampled. Specimens from the NOAA/NMFS slope survey were selected to add additional species and to increase sample sizes.

Enzyme analyses

Examining the difference between pectoral and vertebral WM enzyme activities of the skates in the present study revealed few significant differences between the two muscle types (Table 1). Paired *t* tests show that CS enzyme activity was significantly higher in the pectoral muscle of *A. badia* and *B. microtrachys* (p values <0.05), while MDH showed no trend. LDH and PK tended to be about the same between muscle types. However, LDH and PK activity of *T. californica* was significantly lower in pectoral muscle than vertebral muscle (p value <0.05). Furthermore, this species had statistically lower ratios (pectoral to vertebral) of LDH and CS activity compared with all other species (p values <0.01, ANOVA followed by Tukey post hoc tests). Torpedo rays, unlike skates, utilize their enlarged tails and caudal fins, laterally undulating much like axial undulating sharks (Rosenberger 2001). Due to this, and the difficulty of sampling pectoral locomotor muscle in the juvenile torpedo rays, vertebral muscle for *T. californica* was used in all further analyses, while pectoral muscle was used for all skates.

The effect of differing durations of homogenate centrifugation on CS activity measured was assessed to account for differences in protocol between this and the previous studies. There was a significant linear relationship between the unspun assay and both the quick spun and full spun assays (p <0.001; Fig. 1). The y-intercepts of both the quick spun and full spun correlation reveal that spinning the homogenate reduces CS activity values. The statistically similar slopes and y-intercepts further reveal that the effect of spinning the homogenate occurs with even a 20 s spin. As a result, for comparison to the data, the previously published CS values were adjusted using the equation: unspun CS = 1.29* (full spun CS) + 0.41, where the published data were considered full spun.

Table 1 summarizes the mean activities of CS, MDH, LDH, and PK in WM (vertebral and pectoral) and RM for all species included in the analysis, from the present study and the published literature. Few body mass effects were detected in the species from the present study (data not shown). This is likely the result of small sample sizes and mass ranges for many of the species (Table 1). Due to paucity of significant correlations, comparisons between species were made using means without scaling for body mass.

Depth trends were evaluated in WM and RM enzyme activities using all available data. Enzyme activities were grouped as sharks, rays, and chimaeras due to the decreasing predominance of shark species with increasing depth. Despite marked morphological differences, all three groups fall along the same regression line for WM LDH and PK (Fig. 2). The enzyme activities in RM showed no significant trends with increasing MDO (data not shown). WM glycolytic enzyme activities (LDH and PK) show a statistically significant decline in activity with increasing MDO (Fig. 2). The decline is most pronounced from 0 to 500 m and begins to level off at depths deeper than 1,000 m. WM CS and MDH trends are far less pronounced (Fig. 3). Although CS does not show a statistically significant change with increasing MDO, the two deepest dwelling skates *A. badia* (MDO of 1,585 m) and *B. microtrachys* (MDO of 2,448 m) have very high values (Fig. 3). *A. badia* has a CS activity that is statistically greater than all other species (p value <0.0001,

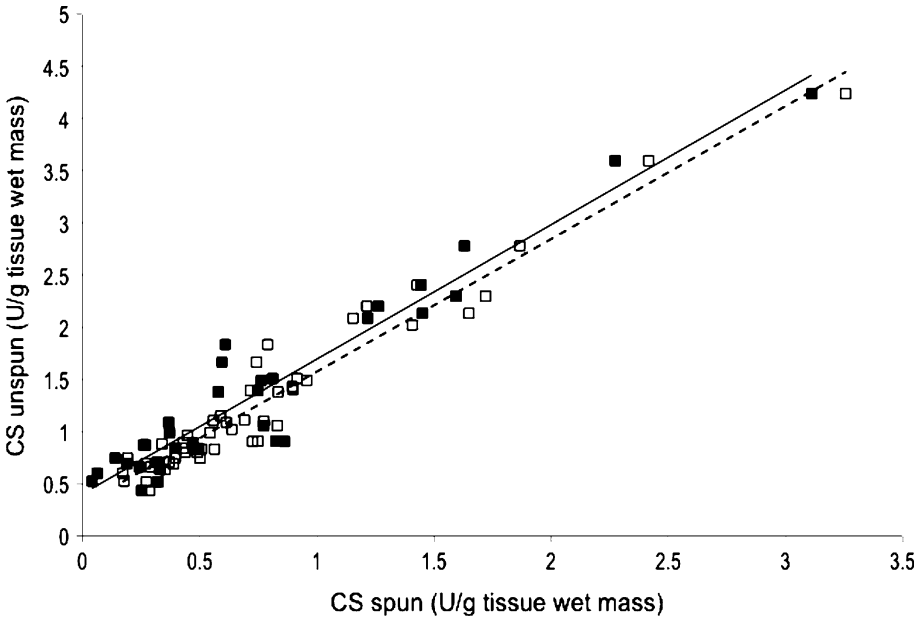

Fig. 1 Relationship between CS activities measured with unspun homogenates, and quick spun (20 s at 5,000 g, *dotted line* and *dash symbols* $y = 1.27x + 0.30$, $r^2 = 0.94$) and full spun (5 min at 5,000 g, *solid line* and *squares* $y = 1.29x + 0.41$, $r^2 = 0.92$) supernatants at 10 °C. Points are assays per individual for the teleost species *Microstomus pacificus*, *Anoplopoma fimbria*, *and Coryphaenoides acrolepis* and the elasmobranch species *Raja inornata*, *Apristurus brunneus*, and *Amblyraja badia*

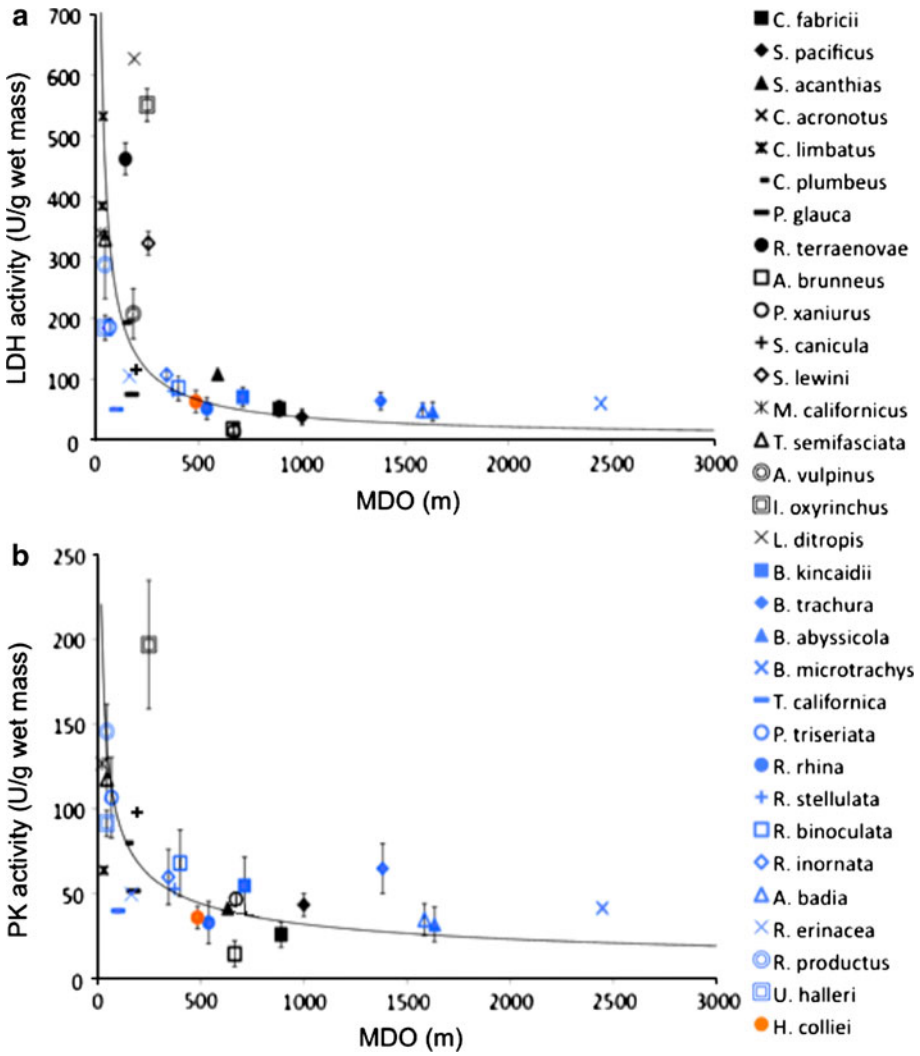

Fig. 2 **a** Mean white muscle (WM) LDH activity (U g^{-1} wet mass) per species (±1 standard deviation) at 10 °C plotted versus median depth of occurrence (MDO) (m). $\log(y) = -0.830*\log(x) + 4.06$ ($r^2 = 0.29$, p value <0.001). **b** Mean WM PK activity (U g^{-1} wet mass) per species at 10 °C plotted versus MDO (m). $\log(y) = -0.460*\log(x) + 2.86$ ($r^2 = 0.2$, p value <0.001). Symbols: *black* sharks; *blue* skates and rays; *orange* chimaera

Kruskal–Wallis followed by Tukey post hoc tests), while *B. microtrachys* is most similar to the species of intermediate depths between 100 and 500 m (Fig. 3). CS activity was further examined using the present study's data exclusively in order to remove any potential bias from adjusted literature data or other procedural inconsistencies. These data, with the exclusion of the two deepest skates, also show no significant change in CS activity with MDO (data not shown). MDH additionally shows no significant change with increasing MDO among all species. There is a significant correlation between WM MDH and CS ($r = 0.55$, p value <0.0001) among all individual fish studied, including the outlying skate species *A. badia* and *B. microtrachys* (Fig. 4). The fact that these skate's values fell in line with the correlation between WM MDH and CS activity values strongly suggests that these activities are not an artifact of experimental error but are truly high.

To examine the effects of phylogeny on enzymatic activities in WM, the species from all available data were grouped by family and by order (Fig. 5). Due to the paucity of MDH and PK data in the previous literature, some species are not represented by activity values of these enzymes. Qualitative comparison among all families revealed that the mean values for CS, LDH, and PK were consistently highest in the family Lamnidae. The family Somniosidae was consistently among the groups with the lowest values for WM CS, MDH, LDH, and PK. Additionally, the two families of skates, Arhynchobatidae and Rajidae, had the highest mean values among those families in which MDH activities have been measured. Families with three or more representative species (Arhynchobatidae, Rajidae, Scyliorhinidae, and

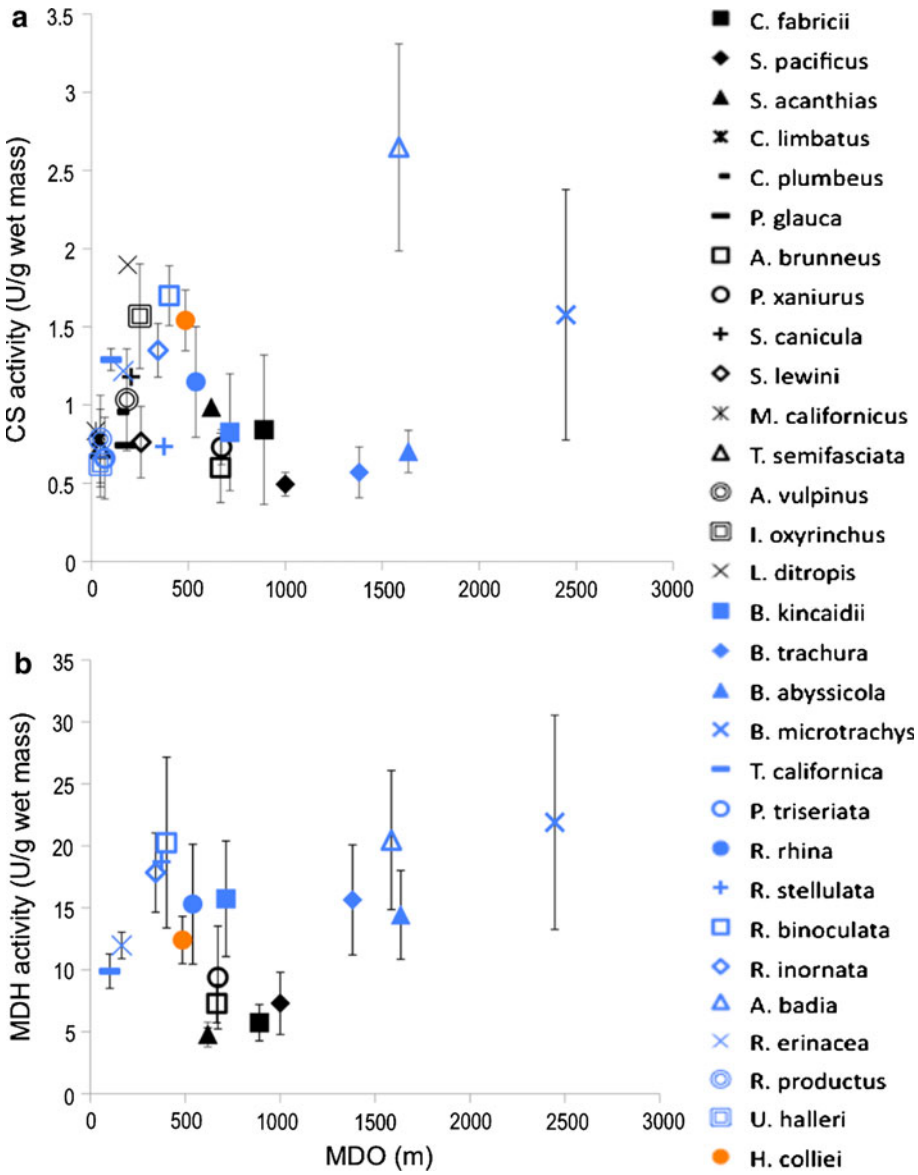

Fig. 3 **a** Mean white muscle (WM) CS activity (U g^{-1} wet mass) per species (±1 standard deviation) at 10 °C plotted versus median depth of occurrence (MDO) (m). **b** Mean WM MDH activity (U g^{-1} wet mass) per species (±1 standard deviation) at 10 °C plotted versus MDO (m). No significant trends were seen. Symbols: *black* sharks; *blue* skates and rays; *orange* chimaera

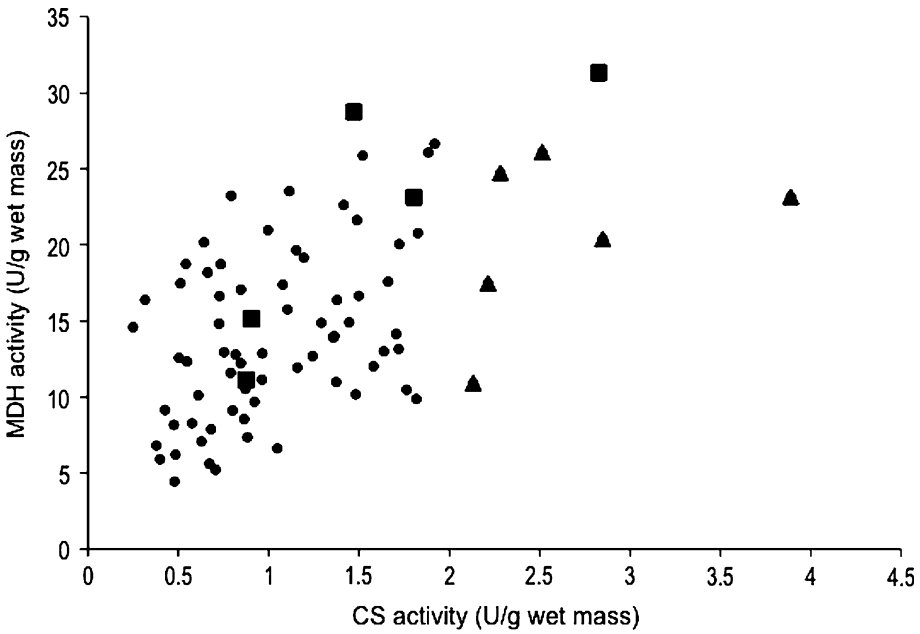

Fig. 4 Correlation of white muscle CS in all fish in the present study. $y = 5.1629x + 8.8758$, r value = 0.55, p value = <0.0001. The two deepest dwelling skates are highlighted: *triangles* = *A. badia* and *squares* = *B. microtrachys*

Carcharhinidae) were examined statistically. Among those families, there existed only two significant differences: carcharhinids had significantly higher LDH activities than all other families (p values <0.01, Kruskal–Wallis followed by Tukey post hoc tests), while the scyliorhinids had significantly lower LDH activities (p values <0.03) than the two skate families. Statistical analyses of species grouped by order (with the exclusion of the single species order Chimaeriformes) revealed no statistically significant difference in WM CS activity. WM PK activity was highest in Lamniformes (p values <0.01). WM LDH activity was more variable, with Lamniformes and Carcharhiniformes having significantly higher values than most other orders. WM MDH activity was significantly higher in the order Rajiformes compared with Carcharhiniformes (two individuals of the family Scyliorhinidae) and Squaliformes (p values <0.05). However, the variability in the range of MDH activities in the Rajiformes was large.

Hierarchical clustering of WM CS and LDH activity data from this and published studies reveals a more complex picture (Fig. 6). The main clusters group from top to bottom with very high WM LDH activity and very high WM CS activity to low LDH and intermediate CS. *Amblyraja badia* is the exception to observed trends, with the highest recorded CS activity and relatively low LDH activity. This species clusters with the endothermic lamnid sharks *Isurus oxyrinchus* and *Lamna ditropis*. At approximately 80 % similarity, three clusters exist for the remaining data. One cluster is comprised of mostly shallow benthic rays and benthopelagic sharks, all of which are statistically indistinguishable from one another. The epipelagic oceanic shark *Alopias vulpinus* is also clustered in this group. The second cluster is composed mainly of shallower dwelling benthic skates, with the exception of the deep-sea skate *B. microtrachys*. The cluster also includes the predominantly shallow benthopelagic sharks *S. acanthias* and *Scyliorhinus canicula*, and the shallow ray *T. californica*. The final cluster is composed of mainly deeper-dwelling benthic skates and benthopelagic squalid and scyliorhinid sharks. This includes one shallower species of skate, *R. stellulata*. The one major exception is the highly migratory, slow-swimming epipelagic shark, *Prionace glauca*. This shark is statistically similar to the squalid *Centroscyllium fabricii* and the skates *B. kincaidii*, *R. stellulata*, and *B. abyssicola*. There are no statistically significant clusters for RM enzyme activities.

Tissue composition

Table 2 summarizes the mean values for protein, water, and lipid contents of each species. The protein ($y = 9.58–0.0012x$, $r^2 = 0.22$, p value = 0.25), water ($y = 79.1 + 0.0014x$, $r^2 = 0.12$, p value = 0.22), and lipid content ($y = 0.74 + 9.89E^{-5}x$, $r^2 = 0.007$, p value = 0.82) showed no significant trend with increasing MDO. *Somniosus pacificus* had significantly greater lipid contents than all other species (p value <0.0001).

Discussion

The present study is the first to examine the metabolic enzyme activities in RM and WM of multiple species of benthic and benthopelagic chondrichthyans over a broad depth range. Significant depth-related declines in two enzyme activities were found, and several interspecific

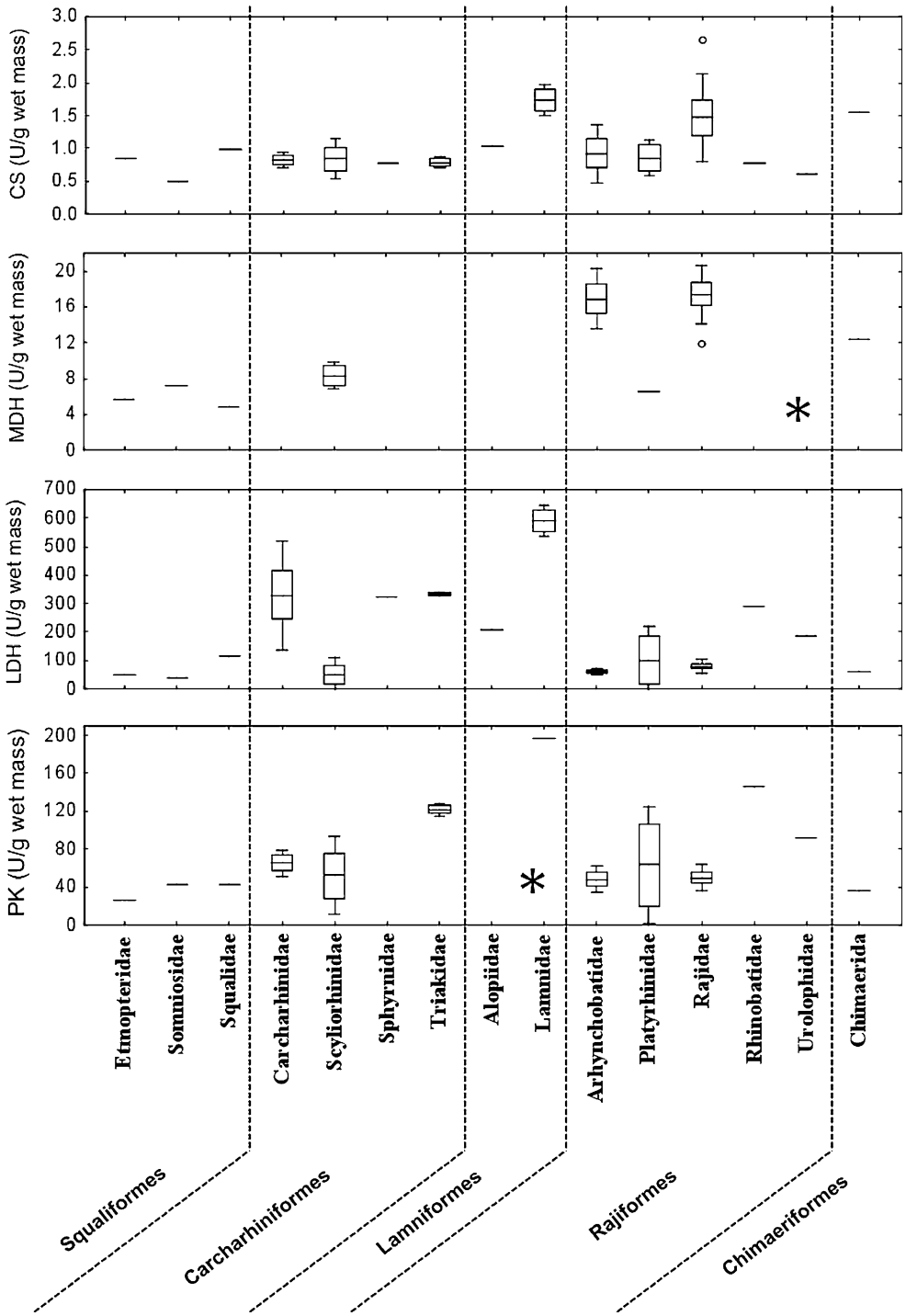

Fig. 5 *Boxplots* of white muscle CS, MDH, LDH, and PK activities (U g^{-1} wet mass, 10 °C) organized by order (Squaliformes, Carcharhiniformes, Lamniformes, Rajiformes, and Chimaeriformes in order *left* to *right*) and family. *Boxplots*: *line* is the mean, *box* is the standard error, *error bars* are standard deviation, and *small circles* signify outliers. The significantly higher PK activities of the order Lamniformes and MDH activities of the order Rajiformes are indicated with an *asterisk symbol*. Families with no data points were not measured

differences appear to correlate with what is known of the ecology of the species studied.

Enzyme activity trends with depth

Across several chondrichthyan species (Table 1), both LDH and PK activities in WM show a significant decline with MDOs ranging from 0 to 1,000 m, with declines most pronounced in the first few hundred meters. After ~1,000 m, activities show little change with depth down to ~2,500 m (Fig. 2). This parallels findings in teleost LDH activity with minimum depth of occurrence (Childress and Somero 1979; Sullivan and Somero 1980; Drazen and Seibel 2007). These pronounced declines in WM glycolytic capacity with increasing depth, in addition to a lack of significant trends with depth in WM CS, WM MDH and

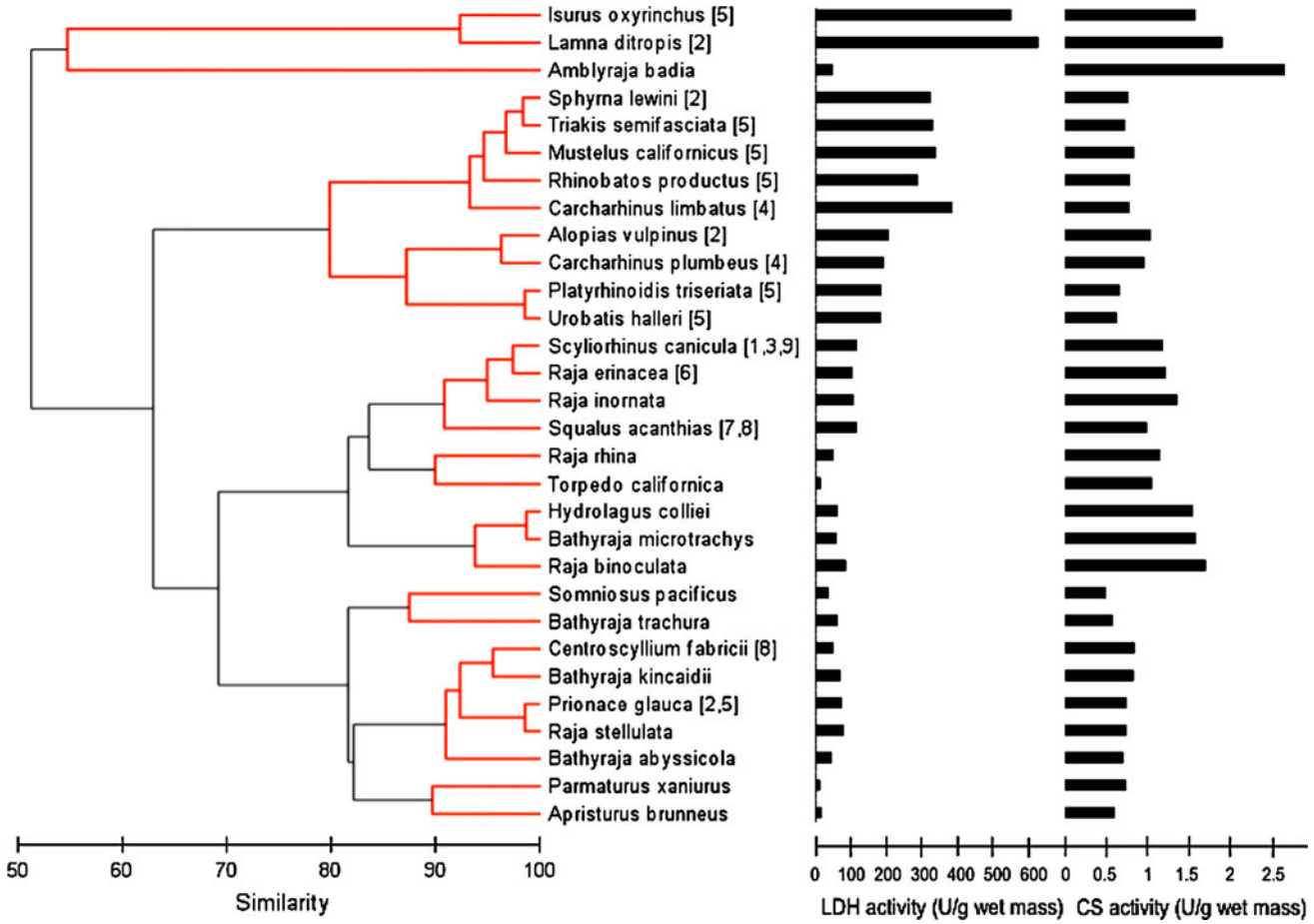

Fig. 6 Hierarchical cluster analysis based on standardized CS and LDH activity values in white muscle at 10 °C from the present and previously published studies. CS values were adjusted according to the equation: unspun CS = 1.29* (full spun CS) + 0.41, where published data were considered full spun. Values were adjusted with a Q_{10} of 2.0 when necessary. *Black lines* represent statistically different clusters, while *red lines* indicate statistically indistinguishable species within clusters. *1* Alp et al. 1976, *2* Bernal et al. 2003, *3* Crabtree and Newsholme 1972, *4* Dickson et al. 1988, *5* Dickson et al. 1993, *6* Moon and Mommsen 1987, *7* Sullivan and Somero 1980, *8* Treberg et al. 2003, *9* Zammit et al. 1978. On the *right* are the mean LDH and CS activity (U g^{-1} wet mass) values for each individual species

Table 2 Mean values for white muscle (vertebral in sharks and rays, and pectoral in skates) % water, protein, and lipid content per species ±1 standard deviation

Species	*n*	% H_20	% protein	% lipid
Apristurus brunneus (brown catshark)	7	84.78 ± 3.32		
Parmaturus xaniurus (filetail catshark)	4	79.26 ± 0.62	8.82 (1)	0.56 (1)
Somniosus pacificus (Pacific sleeper shark)	3	84.12 ± 1.49	4.99 ± 0.48 (2)	2.94 ± 0.18 (2)
Amblyraja badia (broad skate)	6	82.15 ± 0.83	6.78 (1)	0.36 (1)
Bathyraja abyssicola (deepsea skate)	4	80.02 ± 0.38		
Bathyraja kincaidii (sandpaper skate)	8	78.43 ± 0.89 (7)	9.99 ± 0.10 (3)	0.66 ± 0.05 (3)
Bathyraja microtrachys (fine-spined skate)	5	80.61 ± 3.70	7.99 (1)	0.72 (1)
Bathyraja trachura (roughtail skate)	7	80.43 ± 0.44		
Raja binoculata (big skate)	6	77.29 ± 0.44		
Raja inornata (California skate)	5	77.56 ± 0.72		
Raja rhina (longnose skate)	17	78.27 ± 0.89 (15)	10.74 ± 1.32 (9)	0.66 ± 0.08 (9)
Raja stellulata (Pacific starry skate)	1	77.80		
Torpedo californica (Pacific torpedo ray)	2	82.03 ± 0.99	9.41 ± 0.34	0.65 ± 0.05
Hydrolagus colliei (spotted ratfish)	8	79.26 ± 0.86	9.20 ± 1.07 (5)	0.93 ± 0.08 (5)

If sample size was different than the reported "*n*" for a given assay, the number was reported in parentheses next to the value

all four RM enzymes, could be explained by enzyme dilution, the presence of oxygen minimum zones, food limitation, and/or the visual-interactions hypothesis. Each of these factors is discussed to provide insight into the relationship between depth of occurrence and metabolic capacity and potential locomotor capabilities of chondrichthyans.

Enzyme dilution

Although declines with depth were clearly seen in WM LDH and PK activities (Fig. 2), these trends are not likely the result of enzyme dilution (i.e., a lower concentration of enzymes per gram muscle tissue due to comparatively higher water content and lower protein content in deeper-dwelling fishes). In contrast to the previous teleost studies, the present study shows insignificant changes in WM water and protein content with depth for chondrichthyans and little to no effect of enzyme dilution on observed trends. A decline in protein and an increase in water contents with increasing depth have been observed for pelagic teleosts (Childress and Nygaard 1973; Sullivan and Somero 1980; Bailey and Robison 1986; Stickney and Torres 1989; Childress et al. 1990b) and crustaceans (Childress and Nygaard 1974) and are thought to reflect a reduced swimming capacity. However, in the published data, the effect of enzyme dilution was very small as the difference between the water and protein content of shallow- and deep-living species was extremely small in comparison with the difference between WM LDH, MDH, and PK activity. Furthermore, if dilution were the main cause of the observed depth trends, it would be expected that all enzymes studied would show similar trends with depth.

Oxygen minimum zones

Most of the specimens obtained in the present study were caught off the coast of California where waters are characterized by an oxygen minimum zone (OMZ) between approximately 600 and 1,000 m (Levin 2002); however, the data in the present study suggest that the OMZ does not explain the low metabolic enzyme activities of deeper-dwelling chondrichthyans. OMZs have a large regional influence on the abundance, distribution, and physiology of marine organisms (Childress and Seibel 1998; Helly and Levin 2004). Species dwelling within the OMZ have been shown to utilize adaptive strategies such as the suppression of metabolic rates, reduction in locomotor function, and morphological adaptations (e.g., increased gill surface area) for energy conservation (Childress and Seibel 1998; Seibel 2011). If OMZs play a significant part in the reduction in enzymatic activity with depth, one would expect the lowest metabolic enzyme activities in species inhabiting OMZ depths and higher activities in species from greater depths in which oxygen levels exceed those in the OMZ. This is not observed in the present study (Figs. 3 and 4). Similarly, the previous studies have indicated that teleosts show a continued decrease in metabolic enzyme activities with increasing depth despite these low oxygen layers (e.g., Somero and Childress 1980; Drazen and Seibel 2007) and that organisms that live in higher oxygen concentrations below the OMZ have lower metabolic rates (Childress 1995; Childress and Seibel 1998).

Food limitation hypothesis

Based on the existence of an order of magnitude exponential decline in animal biomass with depth, many earlier studies proposed that lower food availability with depth is the driving factor in lower metabolic rates in deep-sea animals (Smith and Hessler 1974; Angel and Baker 1982; Thurston et al. 1994; Dalhoff 2004). However, food supply alone does not explain depth-related declines in metabolic rates. Animals in shallow, oligotrophic waters have metabolic rates higher than animals in deep-sea eutrophic regions (Cowles et al. 1991; Seibel et al. 1997; Seibel and Drazen 2007). If food supply constrained metabolism interspecifically, one would expect these values to be approximately the same. Previous studies additionally show a general lack of correlation between food supply and metabolic rate in the pelagic deep-sea (Seibel and Drazen 2007), as well as a leveling off of WM LDH and CS activities among benthic and benthopelagic fishes after a minimum depth of occurrence of ~500 to 1,000 m (Drazen and Seibel 2007). The data in the present study indicate a similar leveling off of the activities of WM LDH and PK in chondrichthyans past ~1,000 m. If food supply was the major driving factor for the depth-related declines in these enzyme activities then they should continue to decline with depth instead of leveling off.

Visual-interactions hypothesis

The observed depth trends in WM metabolic enzyme activities of chondrichthyans follow closely with the predictions of the VIH. The VIH suggests strong declines in metabolism with depth for species, such as chondrichthyans, that use vision to mediate predator–prey interactions. The pronounced declines in WM glycolytic enzyme activities in the first 1,000 m, and subsequent leveling off from ~1,000 to 2,500 m seen in the present study corroborate this prediction. Similarly, previous teleost studies show a decrease in WM LDH activity with minimum depth of occurrence, with a leveling off of activity after a depth between ~500 and 1,000 m (Childress and Somero 1979; Sullivan and Somero 1980;

Drazen and Seibel 2007). The VIH predicts that these strong declines are the result of a relaxed selective pressure for burst-swimming capacities with decreasing light levels in the first 1,000 m (Childress 1995; Drazen and Seibel 2007). Burst swimming is powered by contraction of the WM tissue using anaerobic glycolysis (Bone 1966; Sullivan and Somero 1980). Therefore, decreases in WM glycolytic enzyme activity should be indicative of decreases in burst-locomotor capacity. The decrease in WM LDH activity, indicative of a decrease in anaerobic capacity, with depth in chondrichthyans in the present study suggests a corresponding decrease in burst-locomotor capacity.

The VIH may also explain the lack of significant declines in RM enzyme activities with depth. The VIH predicts changes in WM enzyme activity and subsequent capacity for burst locomotion with decreasing light levels and reactive distances; however, RM fibers that power sustained slow aerobic swimming (Bone 1966, 1988; Somero and Childress 1980) should remain relatively constant with increasing depth irrespective of changes in ambient visibility and burst-swimming capacity. Such behaviors as daily migratory patterns, foraging, and/or obligate swimming that are powered by RM metabolism are intrinsic daily energetic requirements and should be maintained with depth. For example, deep-living cephalopods have depressed metabolic rates and low mantle muscle enzyme activities at deeper depths, but maintain consistent enzyme activity in their fins and arms that are used for undulatory, slow sustained swimming regardless of depth (Seibel et al. 1997, Seibel and Childress 2000). Interspecific differences in RM metabolic capacity may exist due to other factors, as discussed below, but light level (as a depth correlate) is not an explanatory variable.

In contrast to the WM glycolytic and RM enzyme data, the depth trends seen in WM TCA enzyme activities with depth in chondrichthyans are inconsistent with those observed in the previous studies of teleosts and other animals that have been used to support the VIH. In teleosts, both glycolytic and TCA (MDH and to a lesser extent CS) enzyme activities in WM decrease with depth (Childress and Somero 1979; Sullivan and Somero 1980; Drazen and Seibel 2007). In contrast, despite large decreases in WM glycolytic capacity with depth, chondrichthyans showed no significant depth-related declines in aerobic capacity (Fig. 3). This difference was unexpected, because previous studies have suggested that high CS activities are maintained in WM mitochondria in the presence of high LDH activities in order to facilitate quick post-burst recovery through the rapid processing of accumulated lactate (Dickson 1995, 1996; Gleeson 1996; Bernal et al. 2003). In fishes, lactate generated during burst swimming may be converted to glycogen, oxidized in the WM, or shuttled via the circulatory system to aerobically poised tissues, such as the liver, heart, and gills (Bilinski 1974; Driedzic and Hochachka 1978).

The lack of correlation between WM CS and LDH activities in the present study may be explained by differences in lactate metabolism between chondrichthyans and teleosts. Backey (2007) found no evidence that WM is the site of lactate conversion to glycogen in three shark species (*P. glauca*, *Triakis semifasciata*, and *I. oxyrinchus*), and no correlation between the capacity for lactate processing and the capacity for lactate production within the WM. This contradicts earlier research that indicated retention of lactate in *Squalus acanthias* WM, with the fate of lactate during recovery being gluconeogenesis and glycogen synthesis rather than oxidation (Richards et al. 2003). Although further study is needed, the chondrichthyans in the present study could either simply oxidize all lactate produced with very little conversion to glycogen, or shuttle the majority of lactate into the blood for oxidation or glycogen synthesis in other tissues. In either case, CS in the WM would not be needed to the same extent as it is in teleosts.

Interspecific and phylogenetic comparisons

Among all combined chondrichthyan data (i.e., the present study's data combined with all previously published elasmobranch data), few significant differences in mean WM enzyme activities were found between orders or families suggesting that phylogeny has a small influence on observed decreases in WM anaerobic capacity with increasing depth. Many species of sharks and skates have similar enzyme activity levels when analyzed at comparable temperatures. Conclusions presented in previous studies that show lower enzyme activities in skates and benthic rays compared with species of shallow, active sharks may have been premature due to the relatively few species analyzed (Dickson et al. 1993). Among the four orders compared in the present study, the only indication that shallow, active sharks have higher metabolic enzyme activities than skates was the significantly higher LDH activity in carcharhinids and the significantly higher LDH and PK in Lamniformes compared with the two skate families.

Hierarchical cluster analysis performed using WM CS and LDH activity (representing WM aerobic and anaerobic capacity, respectively) revealed a complex intermixing of phylogenetic groups (Fig. 6). Many of the skates and rays cluster significantly with one or more species of shark that are generally found at similar depth ranges, suggesting similar metabolic capacity in co-occurring demersal sharks and rays.

In addition, general comparison reveals relatively little effect of locomotor mode on observed enzymatic activity

patterns. There is a large diversity of locomotor modes among chondrichthyans (Webb and Keyes 1982; Webb 1993; Donley and Shadwick 2003) that generally cluster among the orders of the species analyzed in the present study. Thus, by statistically analyzing the differences in WM enzyme activities among orders, the effect of locomotor mode on enzymatic activity is analyzed. A lack of clearly established differences in WM CS, LDH, PK, and MDH activity among orders, as well as most families, may indicate a potential adaptation to maximize energetic efficiency in marine environments regardless of body type, locomotor mode, and activity level. This is an additional evidence that enzyme activities are influenced more by MDO than by phylogeny. Examination of a greater number of species within each family is needed to make more accurate comparisons.

Several species stood out in the cluster analysis. The highly migratory epipelagic blue shark, *Prionace glauca*, swims more slowly and less actively than many other pelagic species of carcharhinid sharks (Carey and Sharold 1990). This is corroborated by significantly lower enzyme activities in *P. glauca* relative to other shallow-living carcharhinids. The deeper-dwelling demersal shark *S. pacificus* had among the lowest values measured for CS, PK, MDH, and LDH activity in WM, suggesting low metabolic and locomotor capacity. Two of the three *S. pacificus* were among the largest specimens analyzed in the present study. Due to the small sample size, scaling effects were not factored into these analyses. With larger size, higher LDH and PK activities and lower CS and MDH activities are expected (Somero and Childress 1980). If adjusted for size, the aerobic capacity may be more comparable to smaller sharks and skates, but glycolytic capacity would remain significantly lower than the other species in the present study. *Somniosus pacificus* is generally considered a bottom feeder that may employ lie-in-wait ambush predation to catch large, active prey (Ebert 2003). However, the enzyme data suggest that scavenging might be a common mode to acquire large or active prey due to evidence of low burst-locomotor capacity. Most striking, however, is a significant similarity seen in the cluster analysis between two endothermic lamnid sharks and the deep-water skate *A. badia*. This similarity is driven solely by the relatively high WM CS activity found in this skate. Lamnid sharks regionally conserve heat to maintain above-ambient temperatures in slow-oxidative locomotor muscle (Bernal et al. 2005; Anderson and Goldman 2001). Thus, at in situ muscle temperatures, the muscle enzymatic activity in the lamnid sharks would be far higher than in *A. badia*.

The two deepest dwelling skates in the present study, *A. badia* and *B. microtrachys*, had unexpectedly high WM CS and MDH activity levels (Fig. 3). These high WM aerobic values may be, in part, explained by the idea that WM contributes not only to anaerobic burst swimming, but also to intermediate sustained swimming as proposed by Gruber and Dickson (1997) in leopard sharks. After 6 weeks of endurance training swimming at 60 % of maximal sustainable speeds, there was a 3.6 % increase in WM fiber diameter and a 34 % increase in WM CS activity, indicating that this enzyme is linked to a sustained swimming performance. Both *A. badia* and *B. microtrachys* fell in line with depth-related declines in PK and LDH (Fig. 2). This indicates that these two deep-sea skates, while following observed trends of a decrease in burst-locomotor capacity with depth, may exhibit a higher capacity for some form of sustained swimming behavior compared with the other skates examined. The relatively high values of CS activity in the white pectoral fin muscle compared with the vertebral muscle further suggests some form of sustained, undulatory pectoral fin movement (Table 1). Tagging studies (mark/recapture and telemetry) for Rajidae species have indicated regional home ranges on the order of hundreds of kilometers (Templeman 1984; Hunter et al. 2005; King and McFarlane 2010). Furthermore, Chevolot et al. (2007) proposed that the near absence of genetic differentiation in the skate *Amblyraja radiata* over the entire North Atlantic, compared to regional differences seen in other skates, is indicative of a migratory range much greater than previously thought. The high WM CS and MDH activities seen in the congener *A. badia* and in *B. microtrachys* may therefore reflect a highly migratory behavior on a large horizontal scale.

Conclusions

Continued exploitation of the deep-sea for resources and fisheries, coupled with the potential impacts of global climate change, places an increased importance on understanding the distribution, activity, and energetic demands of understudied deep-dwelling chondrichthyan species. Elasmobranchs are generally considered to be long-lived, late-maturing, low-fecundity species. The little evidence available suggests that these characteristics are accentuated in the deep-sea (Fowler et al. 2005). The present study shows that deep-sea elasmobranchs also have lower locomotor and metabolic capacities in comparison to shallow-water relatives. These trends, predicted by the VIH, are similar to those in teleosts and several other animal groups, providing compelling evidence for this hypothesis. The combination of low metabolic capacity, low growth rates, and low fecundity makes these species highly susceptible to overharvesting and extinction with increasing fishing pressure in the deep-sea.

Acknowledgments We would like to thank reviewers for their thorough comments and input. Specimen collection would not have been possible without the effort of many volunteers: Shaara Ainsley, Mariah Boyle, Donna Kline, Carrie Laxson, Jackie Lighten, Katie Schmidt, Paul Yancey, and John Yeh. A special thanks goes to Dave Ebert and Mariah Boyle for their assistance with species identification at sea. In addition to her support in the field, Michelle Kay provided educational outreach through her website http://deepblusea2009.blogspot.com/. The staff and scientists at the NMFS Northwest Fisheries Science Center provided additional sampling support. We appreciate the time and effort of Mark Bradburn and John Buchanan, as well as the captain and crew of the RV Pt Sur and RV Ms Julie, for their excellent shipboard support. Many thanks also go to undergraduate Erica Aus for assistance with laboratory work. This research was conducted in accordance with University of Hawaii Institutional Animal Care and Use Committee protocols. NSF provided funding for this work through a grant to Jeffrey Drazen (OCE 0727135).

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