Deep-Sea Research I 100 (2015) 117–126

Contents lists available at ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

Enzyme activities of demersal fishes from the shelf to the abyssal plain

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ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form
18 February 2015
Accepted 22 February 2015
Available online 7 March 2015

Keywords:
Metabolism
Visual interactions hypothesis
Enzymes
Locomotory mode

ABSTRACT

The present study examined metabolic enzyme activities of 61 species of demersal fishes (331 individuals) trawled from a 3000 m depth range. Citrate synthase, lactate dehydrogenase, malate dehydrogenase, and pyruvate kinase activities were measured as proxies for aerobic and anaerobic activity and metabolic rate. Fishes were classified according to locomotory mode, either benthic or benthopelagic. Fishes with these two locomotory modes were found to exhibit differences in metabolic enzyme activity. This was particularly clear in the overall activity of citrate synthase, which had higher activity in benthopelagic fishes. Confirming earlier, less comprehensive studies, enzyme activities declined with depth in benthopelagic fishes. For the first time, patterns in benthic species could be explored and these fishes also exhibited depth-related declines in enzyme activity, contrary to expectations of the visual interactions hypothesis. Trends were significant when using depth parameters taken from the literature as well as from the present trawl information, suggesting a robust pattern regardless of the depth metric used. Potential explanations for the depth trends are discussed, but clearly metabolic rate does not vary simply as a function of mass and habitat temperature in fishes as shown by the substantial depth-related changes in enzymatic activities.

1. Introduction

The enormity and inaccessibility of the deep-sea habitat hampers controlled, live-animal experiments like those required for routine measurement of metabolic rate. Despite these difficulties, a number of direct laboratory measurements have been made on deep-sea animals collected mostly from trawls (Ikeda, 2013; Ikeda et al., 2006; Seibel and Drazen, 2007; Seibel et al., 2000; Torres et al., 1979), but also from gentler submersible or ROV capture (Wilson et al., 2013), and a few in situ measurements (Bailey et al., 2002; Drazen and Yeh, 2012; Hughes et al., 2011; Smith, 1978). These few direct measurements of metabolic rate suggest that the rates of some deeper-living animals are lower than those in shallow water and do not conform to predictions based on mass and temperature alone. The observed pattern is an exponential decline in metabolic rates with depth, leveling off at about 1000 m. This trend only occurs in visual taxa (fishes, crustaceans, cephalopods) and does not correlate with food supply. The visual interactions hypothesis (Childress, 1995; Seibel and Drazen, 2007) suggests that metabolism declines with decreasing light levels which result in declining distances over which visually orienting predators and prey can rapidly interact with one another. At the surface, where reaction distances are large, animals maintain high locomotory capability to escape or chase. In darkness, long chases or evasions do not occur and many animals lack streamlining and robust musculature, thus reducing metabolic "overhead."

Given the difficulties of measuring direct metabolic rate in the deep sea, biochemical proxies serve as important estimates. The activities of enzymes in glycolysis and the tricarboxylic acid (TCA) cycle, responsible for the generation of adenosine triphosphate (ATP), approximate the potential metabolic rate of an organism (Childress and Somero, 1979; Dalhoff, 2004; Ombres et al., 2011). Some of these enzymatic activities have correlated directly with metabolic rate or mitochondrial density (Burness et al., 1999; Hochachka and Somero, 2002). Importantly, measurement of their activity can be performed on tissues from recently deceased animals. While these enzyme activities do not provide direct metabolic measurements, they can provide points of interspecific comparison. The use of such proxies is even more valuable for deep-sea fishes, as many have gas bladders that expand, killing fish retrieved from even modest depths.

A comprehensive study of enzyme activities in demersal fishes has not been conducted. Sullivan and Somero (1980) provided the first analysis, showing depth-related declines in enzyme activities down to ~600 m. This analysis, while important, combined benthic and pelagic species and probably does not reflect the complexity of the relationship for demersal animals (e.g. comparing shallow water tuna to deep-sea eelpout). Several other studies focused on specific

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http://dx.doi.org/10.1016/j.dsr.2015.02.013
0967-0637/

families or small groups of species and/or depth ranges (Drazen et al., 2011; Siebenaller et al., 1982; Vetter and Lynn, 1997; Vetter et al., 1994). These often found depth-related trends but not in all cases, perhaps due to a lack of broad depth coverage or low sample sizes (sometimes only one deep and one shallow species; e.g. Treberg et al., 2003; Yang et al., 1992). Important findings from these various studies include similarities in enzyme activities between species of similar foraging habits and locomotory modes. These data were assembled in a meta-analytical approach, though data were limited, particularly for benthic fishes (Drazen and Seibel, 2007), those which spend considerable time resting on the seafloor, such as flatfish. Benthopelagic species, those fishes that are associated with the benthos but spend most of their time swimming in the water column, showed clear depth-related declines in enzyme activities, however trends for benthic species were less certain. Interpretation was complicated because data were acquired from multiple oceans, studies, assay temperatures, and laboratories, each with slightly differing protocols. To provide a stronger evaluation of the factors affecting metabolic enzymatic activity in demersal fishes and reduce uncontrolled variation, a more cohesive data set was needed. Here we present an analysis of enzymatic activity across a broad diversity of 61 species of fish from 50 to 3180 m depth off the central California coast, with a consistent methodology to robustly test whether there are depth-related declines in metabolic enzyme activities.

2. Methods

Demersal fishes were collected primarily by trawl during two oceanographic research expeditions off Monterey Bay, California in 2009. Trawls were conducted between ~50 and 3180 m. Additional specimens were obtained from National Oceanic and Atmospheric Administration trawls in 2009 off the central Oregon coast. The mass and length of the fish were measured. The depths of occurrence of each species were recorded from this sampling effort and also from the literature. Deep-sea fishes display a variety of locomotory modes in the demersal environment and have been very broadly classified as either benthic or benthopelagic (Drazen and Seibel, 2007), the latter term referring to species which spend most of their time swimming in the water column rather than resting on the seafloor. This anecdotal classification is cautiously employed in this study in the absence of any other information.

2.1. Enzymatic assays

White muscle was taken from below the first dorsal fin and inspected carefully to ensure no red muscle was inadvertently sampled. All tissues were frozen in cryovials in liquid nitrogen aboard ship and later stored at −80 °C until the preparation of homogenates for assays. Tissue samples of approximately 0.1 g were homogenized in 1 mL of 0 °C 10 mM Tris/HCl buffer (pH 7.55 at 10 °C). Each fish was examined through the duplicate testing of two independent homogenates.

The maximal activities of four enzymes were assayed using the protocols detailed in Condon et al. (2012). Citrate synthase (CS) catalyzes the first step in the TCA cycle, serves an important role in oxidative metabolism (Somero and Childress, 1980) and correlates with mitochondrial density (Moyes et al., 1992). Malate dehydrogenase (MDH) is a part of the TCA cycle, but also serves to maintain redox balance between the mitochondria and cytoplasm (Gelpi et al., 1992; Ombres et al., 2011; Siebenaller et al., 1982). Lactate dehydrogenase (LDH) is the final enzyme of anaerobic glycolysis, resulting in the production of lactate. Thus, its activity is indicative of anaerobic capacity and, in white muscle, of interspecific burst locomotory capacity (Childress and Somero, 1979; Dalhoff, 2004). Pyruvate kinase (PK) catalyzes the last reaction in glycolysis, producing pyruvate. This substrate can either be used in the mitochondrial TCA cycle or shuttled to anaerobic glycolysis. The rapid flux of pyruvate during anaerobic metabolism suggests that activities of PK more likely indicate anaerobic capacity of the tissue and studies have found positive correlations between PK and LDH in white muscle (Childress and Somero, 1979; Ombres et al., 2011; Sullivan and Somero, 1980).

In assays of these four enzymes the amount of homogenate used was varied to ensure that concentrations of substrates were not limiting. Measurements were made in a spectrophotometer externally cooled to 10 °C. Activity levels are expressed as μmoles product generated per minute (SI unit) per gram wet weight of tissue). Specific reaction conditions may be referenced from Condon et al. (2012).

2.2. Water content

Subsamples of white muscle taken during initial collection were used to conduct water content assays. Tissue samples were initially weighed, dried for 24 h in a 60 °C furnace, and then weighed again. Care was taken to constrain condensation or rehydration. Each fish was tested in triplicate, and the resulting mean difference in weight was attributed to the water content of the tissue and recorded as a percentage relative to the overall tissue composition.

2.3. Depth parameterization

To evaluate depth patterns, mean enzymatic activities of each species were regressed against depth. For pelagic species which undergo diel vertical migration, minimum depth of occurrence has been used in previous studies. This characterization of species habitat was chosen because the minimum depth represents the extreme end of the individual's daily range in food and light levels. Many demersal fishes exhibit ontogenetic downslope migrations (Jacobson et al., 2001; Polloni et al., 1979; Yeh and Drazen, 2011). Little is known about whether diel vertical migration occurs up- and down-slope. Therefore, depth ranges (for adults) often represent a broader range than for pelagic species, one that individual fish likely do not use on a daily basis. Further, the minimum depth of occurrence has been argued to represent only a small portion of a demersal species population, perhaps making median depth of occurrence a more appropriate parameter (Condon et al., 2012). For this study we decided to conduct analyses on minimum, median, and maximum depths of occurrence to fully evaluate habitat influences on metabolic enzyme activities. Further we used our own trawl capture information and known depth ranges from the literature which can encompass a broader range than any single sampling effort.

2.4. Size effects

Body mass affects metabolic enzymatic activities in fishes and other animals (Childress and Somero, 1990; Seibel, 2007; Somero and Childress, 1980). These relationships are usually power functions. Thus, we examined the relationship between body mass and enzyme activity for each species using linear regression after first performing natural log transformations of the data. This was done for all species where there were more than 4 individuals. With the great diversity of species examined there was a wide range of body sizes, and the size ranges of many species did not overlap (Table 1). This made the use of intraspecific enzyme-body mass regressions to standardize enzyme activities to a mean size difficult to justify for interspecific comparisons. Even when significant relationships were found, they would require extrapolation of enzyme activities to outside the natural range of body mass for many species, certainly outside the size range of specimens from the present analysis.

Table 1
Demersal fish species' depth and size ranges and enzyme activities. Mean enzyme activities were measured at 10 °C and are presented with ±1 standard deviation. Minimum and maximum depth ranges are presented with literature references. Locomotory mode is notated as B—benthic or BP—benthopelagic. CS—citrate synthase, MDH—malate dehydrogenase, LDH—lactate dehydrogenase, PK—pyruvate kinase. Depth references are indicated as Anderson (1995) (A); Anderson and Peden (1988) (B); Clausen (2008) (C); Cohen et al. (1990) (D); Hoff et al. (2000) (E); Jacobson and Vetter (1996) (F); Jacobson et al. (2001) (G); Lauth (2000) (H); Love et al. (2002) (I); Miller and Lea (1972) (J); Pearcy et al. (1982) (K); Stein et al. (2006) (L); Wilson and Waples (1983) (M); Yeh and Drazen (2011) (N); this study (+).

Species	*n*	Depth (m)			Mass (g)			Enzyme activity (units g^{-1})			
		Trawl range	Min.	Max.	Range	Mean	Loc. mode	CS	MDH	LDH	PK
Order Batrachoidiformes											
Family Batrachoididae											
Porichthys notatus	5	87–216	10 (J)	300 (J)	19–119	55	B	1.27 ± 0.31	14.14 ± 4.43	40.9 ± 6.9	25.88 ± 8.06
Order Gadiformes											
Family Macrouridae											
Albatrossia pectoralis	5	1230–1347	565 (H)	1300 (C,J)	551–6555	3047	BP	0.16 ± 0.09	4.68 ± 1.18	20.1 ± 7.1	9.17 ± 3.2
Coryphaenoides acrolepis	15	970–2165	730 (H)	2000 (J)	16–2711	894	BP	0.85 ± 0.43	16.47 ± 6.65	99.5 ± 75.6	16.11 ± 5.79
Coryphaenoides armatus	6	2000–3066	1998 (+,K)	4100 (M)	52–2769	926	BP	1.77 ± 0.92	20.85 ± 10.5	74.1 ± 47.5	23.09 ± 13.0
Coryphaenoides filifer	7	1980–3130	1975 (+,K)	3050 (+,K)	86–4330	1005	BP	0.39 ± 0.27	17.72 ± 18.0	53.1 ± 23.5	21.01 ± 4.09
Coryphaenoides leptolepis	5	2075–3181	1998 (K)	3858 (K)	50–400	199	BP	0.98 ± 0.49	13.56 ± 2.24	11.7 ± 3.60	29.9 ± 7.37
Nezumia liolepis	5	715–794	581 (E)	1247 (E)	7.4–80	46	BP	1.7 ± 0.45	16.60 ± 8.43	20.6 ± 10.0	23.96 ± 6.97
Family Merlucciidae											
Merluccius productus	5	133–570	45 (D)	500 (D)	31–673	339	BP	2.27 ± 0.49	26.10 ± 5.65	108.7 ± 61.1	40.26 ± 19.5
Family Moridae											
Antimora microlepis	6	970–2374	510 (H)	2320 (+)	28–1693	799	BP	0.66 ± 0.44	10.99 ± 2.34	27.3 ± 13.2	20.49 ± 5.47
Order Ophidiiformes											
Family Ophidiidae											
Chilara taylori	5	201–220	10 (J)	250 (+,J)	8.0–70	35	B	1.53 ± 0.39	20.34 ± 3.82	124.4 ± 32.8	60.03 ± 11.8
Spectrunculus grandis	3	2000–3150	2130 (K)	3144 (K,N)	607–17463	6865	BP	0.21 ± 0.08	9.29 ± 5.21	93.8 ± 31.5	36.09 ± 9.75
Order Osmeriformes											
Family Alepocephalidae											
Alepocephalus tenebrosus	5	715–2165	550 (H)	2040 (+)	30–2436	1064	BP	0.32 ± 0.2	6.59 ± 4.55	35.2 ± 26.4	13.61 ± 5.63
Rouleina sp.	1	2105–2296	2105 (+)	2296 (+)	351	351	BP	0.18 ± 0.07	11.01 ± 1.16	56.1 ± 16.2	25.27 ± 2.62
Order Perciformes											
Family Embiotocidae											
Zalembius rosaceus	6	87–117	10 (J)	92 (J)	21–52	35	BP	3.15 ± 2.02	37.24 ± 10.7	153.8 ± 48.8	55.56 ± 20.1
Family Sciaenidae							BP				
Genyonemus lineatus	5	87–117	10 (J)	102 (J)	113–240	183	BP	2.26 ± 0.38	30.34 ± 6	263.9 ± 61.5	114.1 ± 25.4
Family Zoarcidae											
Bothrocara brunneum	5	970–2250	466 (H)	2320 (+)	607–890	754	BP	0.13 ± 0.11	4.84 ± 1.63	21.6 ± 7	10.22 ± 3.4
Bothrocara molle	11	1980–2374	2000 (+,K)	2688 (K)	15–72	41	BP	0.29 ± 0.21	5.75 ± 2.16	19.5 ± 7.1	9.93 ± 2.39
Lycenchelys micropora	2	1980–2350	2377 (A)	3512 (A)	55–124	90	B	0.54 ± 0.46	9.31 ± 2.41	109 ± 39.6	29.89 ± 10
Lycenchelys sp *A*	5	2105–2374	2105 (+)	2374 (+)	36–107	71	B	0.34 ± 0.22	10.92 ± 3.9	101.1 ± 22.3	29.44 ± 7.51
Lycenchelys sp *B*	6	2780–3150	2780 (+)	3150 (+)	23–158	84	B	0.57 ± 0.35	10.97 ± 7.53	135 ± 93.2	58.35 ± 32.1
Lycodes cortezianus	5	133–570	92 (J)	549 (H)	13–244	119	B	1.07 ± 0.2	16.50 ± 4.26	116.2 ± 20.2	58.86 ± 17.8
Lycodes diapterus	6	410–1133	300 (H,+)	983 (H)	7.2–81	42	B	0.68 ± 0.36	17.03 ± 6.93	142.4 ± 52.9	55.73 ± 21.8
Lyconema barbatum	2	201–271	83 (J)	376 (J)	2.5–12	8.0	B	1.82 ± 1.15	28.77 ± 12.4	344.7 ± 83.2	75.89 ± 21.2
Pachycara bulbiceps	3	2780–3150	2600 (B)	4000 (B)	48–92	70	B	1.1 ± 0.36	9.45 ± 3.32	68.9 ± 20.1	23.54 ± 6.33
Pachycara gymninium	5	2780–3150	1829 (B)	3219 (B)	140–901	457	B	0.58 ± 0.22	17.48 ± 9.38	126.2 ± 47.8	32.12 ± 10.2
Pachycara karenae	1	2780–3150	2780 (+)	3150 (+)	377–728	512	B	0.26 ± 0.12	8.2 ± 3.32	38.3 ± 27.4	26.02 ± 17.7
Pachycara lepinium	5	2105–2296	1728 (B)	2970 (B)	491	491	B	0.23 ± 0.07	10.29 ± 3.77	78.3 ± 13.7	30.60 ± 3.07
Order Pleuronectiformes											
Family Cynoglossidae											
Symphurus atricaudus	3	80–117	10 (J)	85 (J)	14–37	25	B	0.42 ± 0.24	22.25 ± 5.63	212.1 ± 89.8	50.22 ± 25.3
Family Paralicthyidae											
Citharichthys sordidus	17	73–89	10 (J)	227 (H)	20–220	113	B	0.94 ± 0.36	41.97 ± 9.25	178.8 ± 52.7	54.41 ± 17.1
Family Pleuronectidae											
Astheresthes stomias	1	446–483	189 (H)	589 (H)	371.4	371	B	0.62 ± 0.02	9.32 ± 0.88	52.8 ± 4.4	17.79 ± 4.29
Embassichthys bathybius	11	792–1347	732 (H,K)	1430 (K)	88–1016	755	B	0.24 ± 0.15	10.94 ± 8.02	77.5 ± 33.4	34.55 ± 29.1
Eopsetta jordani	5	88–217	20 (J)	465 (H)	86–1071	450	B	0.66 ± 0.19	12.34 ± 5.97	178.9 ± 65.6	67.14 ± 22.9

Table 1 (*continued*)

Species	*n*	Depth (m)			Mass (g)			Enzyme activity (units g^{-1})			
		Trawl range	Min.	Max.	Range	Mean	Loc. mode	CS	MDH	LDH	PK
Glyptocephalus zachirus	8	88–570	60 (J)	500 (H)	15–483	165	B	0.65 ± 0.26	8.88 ± 4.38	102.4 ± 37.4	33 ± 8.7
Lyopsetta exilis	5	88–233	77 (J)	366 (H)	9.5–63	39	B	0.73 ± 0.18	11.09 ± 4.99	127.1 ± 60.7	37.76 ± 10.1
Microstomus pacificus	15	111–1347	50 (+,J)	1100 (G)	20–1611	586	B	0.84 ± 0.87	14.49 ± 7.98	111.5 ± 61.4	53.33 ± 22.7
Parophrys vetulus	20	80–140	20 (J)	408 (H)	22–340	136	B	1.12 ± 0.46	22.55 ± 8.34	108.1 ± 77.3	52.47 ± 18
Order Scorpaeniformes											
Family Agonidae											
Bathyagonus pentacanthus	4	87–89	50 (+,J)	454 (H)	12–186	99	B	1.32 ± 0.28	16.41 ± 3.5	366.0 ± 91.6	154.3 ± 21.1
Family Anoplopomatidae											
Anoplopoma fimbria	12	87–1330	190 (H)	1290 (G)	103–3825	1677	BP	1.59 ± 1.08	20.86 ± 12.4	166.6 ± 50.2	40.05 ± 25
Family Cottidae											
Chitonotus pugetensis	1	88–89	10 (J)	140 (J)	15	15	B	2.11 ± 0.3	50.44 ± 9.32	403.6 ± 159.	217.3 ± 12.3
Icelinus tenuis	1	80–82	30 (J)	375 (J)	18	18	B	2.73 ± 0.12	53.39 ± 6.47	435.8 ± 87.7	176.1 ± 15.4
Leptocottus armatus	11	73–74	10 (J)	102 (J)	100–200	136	B	2.43 ± 0.44	34.69 ± 7.67	156.7 ± 48	52.5 ± 12.6
Family Hexagrammidae											
Ophiodon elongatus	5	87–140	10 (J)	408 (H)	26–162	68	B	2.04 ± 0.36	22 ± 5	120.3 ± 38.6	28.86 ± 5.87
Zaniolepis latipinnis	4	87–89	37 (J)	114 (J)	22–50	36	B	1.21 ± 0.36	26.02 ± 7.69	302.8 ± 80.9	110.7 ± 32.6
Family Liparidae											
Careproctus cypselurus	1	970–1024	817 (H)	1400 (H)	48	48	BP	1.54 ± 0.25	12.61 ± 1.6	18 ± 5.4	13.97 ± 1.59
Careproctus gilberti	1	2040–2165	632 (L)	2040 (+)	41	41	BP	1.48 ± 0.06	17.77 ± 1.9	25.4 ± 2.6	20.75 ± 3.58
Careproctus melanurus	7	476–1347	464 (+,L)	1280 (+,L)	13–193	80	BP	1.26 ± 0.54	16.82 ± 5.71	29 ± 19	20.07 ± 4.66
Family Sebastidae											
Sebastes aurora	1	464–528	300 (I)	500 (I)	312	312	BP	0.59 ± 0.01	13.64 ± 2.69	42.4 ± 2.3	19.86 ± 1.77
Sebastes caurinus	1	100–100	10 (I)	183 (I)	1956	1956	BP	0.56 ± 0.08	39.02 ± 9.6	495.8 ± 36.5	173.7 ± 14.8
Sebastes chlorostictus	1	111–117	30 (I)	363 (I)	13	13	BP	3.46 ± 0.32	21.39 ± 5.21	104.1 ± 15.8	28.84 ± 1.98
Sebastes crameri	1	256–271	140 (I)	210 (I)	393	393	BP	0.65 ± 0.13	25.00 ± 0.52	136.1 ± 15.1	37.82 ± 4.62
Sebastes diploproa	10	133–570	200 (I)	600 (I)	22–599	336	BP	1.16 ± 1.02	16.02 ± 7.36	51.3 ± 33.3	18.04 ± 15.7
Sebastes elongatus	4	88–216	100 (I)	250 (I)	23–95	43	BP	2.46 ± 0.55	42.13 ± 7.04	275.1 ± 54.2	65.68 ± 10.6
Sebastes goodei	5	133–271	75 (I)	325 (I)	78–1154	490	BP	3.74 ± 0.43	23.93 ± 9.44	325.9 ± 70.7	128.2 ± 25.1
Sebastes jordani	5	133–258	150 (I)	200 (I)	28–129	70	BP	3.49 ± 0.85	52.28 ± 11.1	423.2 ± 155	107.8 ± 50.1
Sebastes levis	1	88–89	40 (I)	491 (I)	15	15	BP	1.56 ± 0.13	12.07 ± 1.41	142.1 ± 5.9	42.84 ± 5.65
Sebastes melanostoma	2	436–610	200 (I)	600 (I)	328–646	487	BP	0.45 ± 0.1	5.73 ± 0.82	53.9 ± 8.5	23.4 ± 3.78
Sebastes proriger	1	201–216	150 (I)	275 (I)	201	201	BP	3.19 ± 0.18	16.06 ± 0.92	132.9 ± 17.2	57.89 ± 4.92
Sebastes saxicola	5	50–258	100 (I)	200 (I)	19–25	22	BP	2.58 ± 0.34	22.29 ± 8.35	216.3 ± 82.5	66.36 ± 20.5
Sebastes semicinctus	5	50–570	60 (I)	150 (I)	38–58	48	BP	2.71 ± 0.52	38.90 ± 8.03	451.3 ± 98	133.4 ± 24.1
Sebastolobus alascanus	10	324–1347	300 (F,I)	1200 (F,I)	20–3565	1259	B	0.47 ± 0.21	7.01 ± 2.5	63.9 ± 15.2	20.52 ± 6.19
Sebastolobus altivelis	11	464–1480	500 (F,I)	1400 (F,I)	25–271	188	B	0.46 ± 0.18	6.16 ± 2.25	58.9 ± 25.2	11.34 ± 7.65

Therefore, we used a generalized linear model which included the mean size of each species as a variable, to allow interspecific size effects to be evaluated (see Section 2.5).

2.5. Statistical analysis

We evaluated the effect of collection time (seasons—all in 2009) on enzymatic activities and found no significant effects (PERMANOVA, $p > 0.05$) so this factor was removed from subsequent analyses. A generalized linear regression model was used to evaluate the effects of depth and body mass (both continuous), and general locomotory mode (categorical predictor). To achieve normal distributions, depth and body mass were natural log transformed prior to analysis. An identity link function was used to parameterize the relationship between enzyme activity and continuous predictors in the model. As the main goal of the study was to evaluate depth effects, the model was used to predict each species enzyme activities as a function of depth after taking into account any significant effects of body size and locomotory mode.

3. Results

The rates of CS, MDH, LDH and PK were analyzed for a total of 61 species of demersal fishes comprised of 335 individuals (Table 1; Fig. 1). Of these, 29 species (182 individuals) were classified as benthic and 32 species (153 individuals) as benthopelagic. Specimens were collected from between 50 and 3180 m depth. Literature habitat depths ranged from sub-tidal to about 4100 m on the abyssal plain off California (see Table 1 for references).

Seventeen species had sample sizes large enough ($n > 4$) to test for body mass effects on enzymatic activity and in each case there was at least one significant relationship (Table 2). For many species, no significant relationships were found, but this may be the result of relatively low sample sizes or narrow size ranges for individual species (Table 1). TCA cycle enzymes (CS and MDH) scaled negatively with body mass, where the relationship takes the form: enzyme activity (units g^{-1} wet mass)$=a*$mass $(g)^b$. For CS most scaling exponents ranged from -0.25 to -0.56. The exceptions were the California tonguefish, *Symphurus atricauda* ($b=-1.94$) and the rosy surfperch, *Zalembius rosaceus* ($b=-1.54$), both of which had relatively small sample sizes ($n=5$ and 6, respectively). Exponents for MDH ranged from -0.14 to -0.32. Scaling exponents for glycolytic enzymes (LDH and PK) were highly variable (-0.69 to 0.46). Both species that exhibited positive relationships between body mass and either LDH or PK were Gadiformes. Interspecifically, the fishes examined spanned a wide size range, 2.5–6555 g, with one outlier, a 17.5 kg giant cuskeel *Spectrunculus grandis* (Table 1). Mean mass was 466 g (median 120 g). Body mass was a significant predictor for CS, with negative quarter power scaling, but did not have a significant effect on the other enzyme activities (Table 3).

There were significant differences in some of the enzyme activities between benthic and benthopelagic species (Table 3). The GLM analysis using six variations of the depth parameter (minimum, median, and maximum for both trawl and literature values) showed consistent differences in CS activity as a function of locomotory mode ($p < 0.01$; Table 3) with benthopelagic species having higher values than benthic species (Fig. 1). MDH values were only significantly higher in benthopelagic species using minimum literature depths ($p < 0.05$). LDH was significantly higher in benthic species (positive parameter estimates; Table 3) for a few of the depth parameters.

The strongest predictor of all four enzymatic activities was depth of occurrence (Table 3; Fig. 1). The depth parameter used had no influence on the significance of the result. Indeed, with the exception of minimum literature depth, the parameter estimates for the depth effect were quite consistent regardless of which depth parameter was used. In all cases the parameter estimate was smaller for minimum literature depth suggesting a less pronounced decline in enzymatic activity with minimum depth of occurrence.

A few orders or families of fishes examined in this study included many different species with members sharing similar morphology and ecology allowing for within-family examination of depth effects (Table 4). The Gadiformes included mostly macrourids, one merluccid and one morid (Table 1). All are elongate, benthopelagic fishes. Both CS and PK scaled negatively with body mass interspecifically. While depth had no significant effect, there was only a single species, *Merluccius productus*, shallower than 500 m (Table 1). The Perciformes studied exhibit high diversity in body form and habits. Within the family Zoarcidae, there were representative species across a broad depth range, including benthic and two benthopelagic species. The two benthopelagic fishes, *Bothrocara brunneum* and *Bothrocara molle*, had significantly lower enzyme activities than the rest of the species which are benthic (Table 4). Mass scaled negatively to CS and LDH in half of the cases. LDH, MDH, and PK declined with depth across the Zoarcid species in nearly all scenarios, but CS did not show significant declines with depth. The Pleuronectiformes, benthic flatfishes, had many representatives that reached depths of about 1500 m. Declines with depth in CS, LDH, and PK were significant for minimum depths and in a few cases for median depths (Table 4). The Scorpaeniformes are as diverse as the Perciformes in our data set, including snailfish (Liparidae), large predatory species such as the lingcod (*Ophiodon elongatus*), and small armored Agonids. However, the family Sebastidae, which include rockfishes, are very similar in body form and ecology. The genus *Sebastolobus* differs from *Sebastes* in that its members are benthic, lack a gas bladder, and are not viviparous so only the genus *Sebastes* was used for GLM analysis. For the genus *Sebastes*, mass scaled negatively with CS activities, and depth-related declines were significant for LDH, MDH and PK when using approximately half of the depth metrics (Table 4).

Water content of the white muscle tissue increased with depth in the benthopelagic species ($p < 0.05$). In the benthic species water content showed no significant trend with depth (Fig. 2).

4. Discussion

Specific enzyme activities were generally comparable to previously published values. Interspecific scaling relationships were also similar to those first identified by Somero and Childress (1980), as well as some reported later (Vetter and Lynn, 1997; Vetter et al., 1994). The citrate synthase values found in the present study are higher than previously reported values for rockfish (Vetter and Lynn, 1997) and some macrourids (Siebenaller et al., 1982). This is likely the result of methodological differences as reported by Condon et al. (2012), but does not affect the relative values. It does emphasize the need for consistent methodology and illustrates the potential problems with early meta-analytic approaches (Drazen and Seibel, 2007).

This study reaffirmed depth-related declines in the metabolism of fishes independent of temperature and body size. The declines are not due to a simple increase in the proportion of water in the muscle. Though an increase in water content with depth was evident for benthopelagic (but not benthic) species as noted in other studies (Crabtree, 1995) it was not of a sufficient magnitude to explain the 3 to 5 fold declines in enzymatic activities with depth (Sullivan and Somero, 1980). Importantly, the declines in enzymatic activities were significant and robust regardless of the depth parameter used in our analyses (capture depths and published depth range metrics). This suggests that a pattern exists which is relatively insensitive to the different depth metrics, despite sometimes pronounced ontogenetic changes in habitat depth or movements of individuals within their broad depth ranges. The present

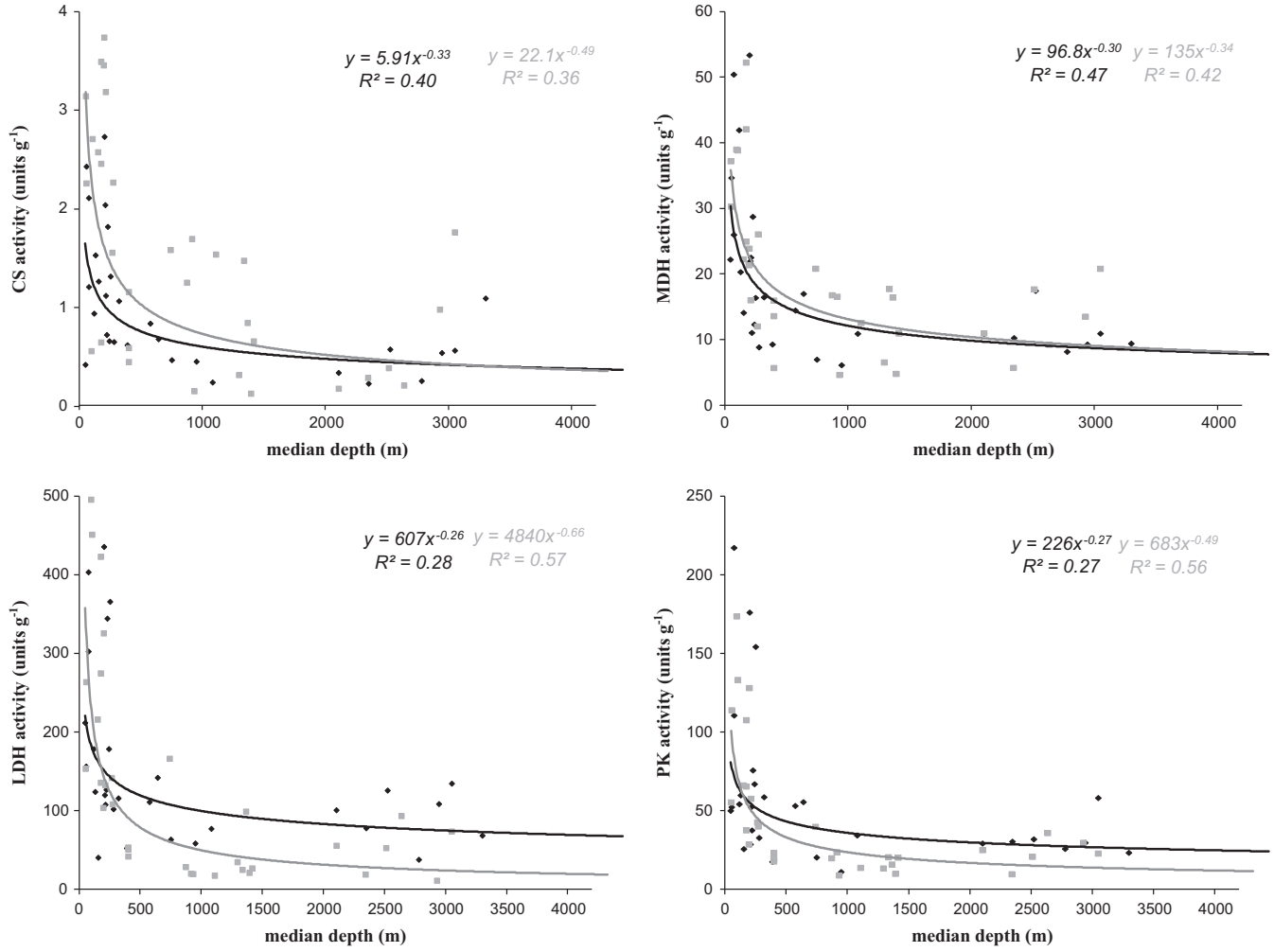


Fig. 1. Enzymatic activities (units g^{-1} wet mass at 10 °C) as a function of median depth of occurrence in benthic (black diamonds) and benthopelagic (gray squares) species.

Table 2
Enzyme mass scaling relationships (power functions) by species. Equations take the form of enzyme activity (units g^{-1} wet weight)=a*mass (g)^b. Functions are presented if significant ($p < 0.05$).

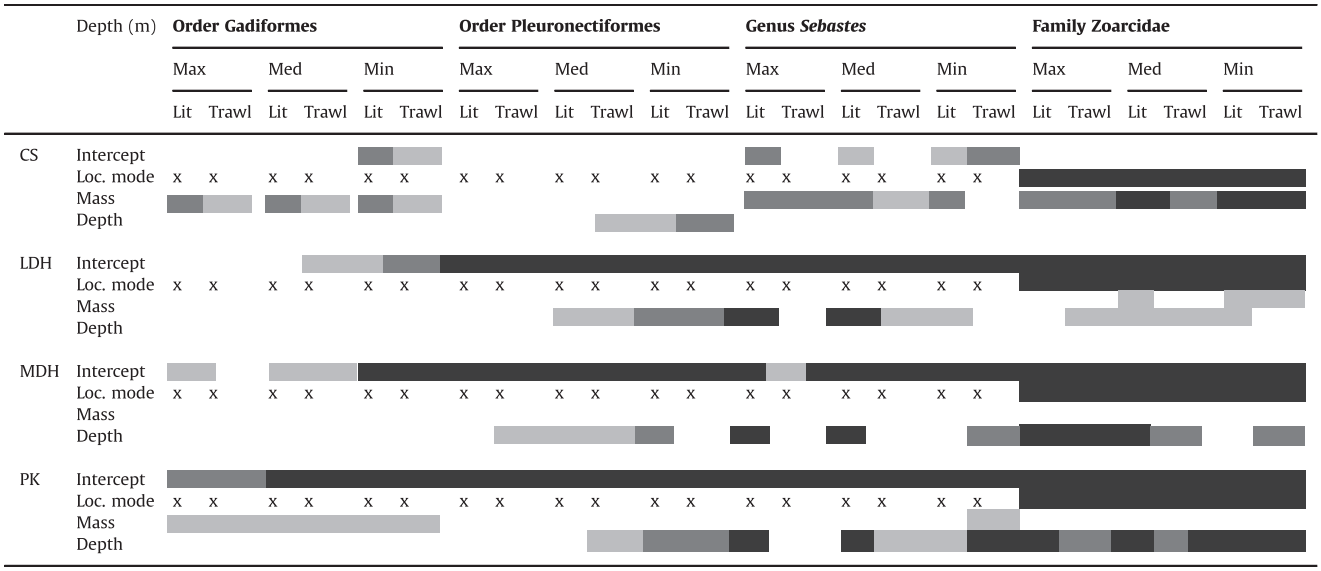
Order	Species	N	CS		MDH		LDH		PK	
			$f(x)$	R^2	$f(x)$	R^2	$f(x)$	R^2	$f(x)$	R^2
Gadiformes	*Antimora microlepis*	6	$6.41x^{-0.40}$	0.69	–	–	–	–	–	–
	Coryphaenoides acrolepis	15	$3.93x^{-0.28}$	0.61	–	–	$5.88x^{0.40}$	0.44	–	–
	Coryphaenoides leptolepis	7	$16.2x^{-0.58}$	0.83	–	–	–	–	–	–
	Merluccius productus	5	–	–	–	–	$8.06x^{0.46}$	0.84	$7.16x^{0.31}$	0.79
Perciformes	*Lyconema barbatum*	6	$8.40x^{-0.88}$	0.69	–	–	–	–	–	–
	Zalembius rosaceus	6	$605x^{-1.54}$	0.74	–	–	–	–	–	–
Pleuronectiformes	*Embassichthys bathybius*	11	–	–	–	–	$1850x^{-0.51}$	0.55	$2850x^{-0.69}$	0.49
	Glyptocephalus zachirus	8	$2.01x^{-0.26}$	0.68	–	–	–	–	–	–
	Lyopsetta exilis	5	$1.99x^{-0.30}$	0.92	–	–	–	–	–	–
	Microstomus pacificus	15	–	–	$31.8x^{-0.15}$	0.35	$503x^{-0.30}$	0.60	–	–
	Parophrys vetulus	20	$3.34x^{-0.25}$	0.26	–	–	–	–	–	–
	Symphurus atricauda	5	$164x^{-1.94}$	0.90	–	–	–	–	–	–
Scorpaeniformes	*Sebastolobus alascanus*	10	–	–	$16.5x^{-0.14}$	0.53	–	–	–	–
	Sebastolobus altivelis	11	–	–	$20.9x^{-0.24}$	0.40	–	–	–	–
	Anoplopoma fimbria	12	$40.8x^{-0.51}$	0.74	$163x^{-0.32}$	0.56	–	–	–	–
	Sebastes diploproa	10	$20.3x^{-0.56}$	0.59	–	–	–	–	$144x^{-0.40}$	0.52
	Sebastes jordani	5	–	–	–	–	$3650x^{-0.55}$	0.86	$1280x^{-0.64}$	0.82

results corroborate earlier studies that were based upon more limited data (Drazen and Seibel, 2007; Sullivan and Somero, 1980) and parallel respirometry data which show that deep-sea benthopelagic fishes such as macrourids, morids, and ophidiids have 10-fold lower metabolic rates than shallow living species (Drazen and Yeh, 2012; Smith, 1978). The depth pattern is also similar to those found in sharks and skates collected concurrently with the teleosts studied here (Condon et al., 2012). For the elasmobranchs, phylogeny and locomotory mode (sharks with caudal swimming versus skates with pectoral fin swimming) had little influence on enzyme

Table 3
Generalized linear model results for each enzyme activity. Parameter estimates and significance ($p < 0.05$ – underlined; $p < 0.01$ – light grey shading, $p < 0.001$ – dark grey shading) are given with standard error. Models are fit to maximum, median, and minimum depth estimates for species using empirical trawl data from the present study and from the literature.

Depth parameter		Max Lit	Max Trawl	Med Lit	Med Trawl	Min Lit	Min Trawl
CS	Intercept	3.38 ± 0.48	3.19 ± 0.43	3.17 ± 0.42	3.26 ± 0.41	2.55 ± 0.33	3.2 ± 0.37
	Loc. mode	−0.46 ± 0.16	−0.51 ± 0.16	−0.47 ± 0.16	−0.51 ± 0.15	−0.57 ± 0.16	−0.51 ± 0.15
	Mass	−0.25 ± 0.06	−0.23 ± 0.06	−0.25 ± 0.06	−0.23 ± 0.06	−0.27 ± 0.06	−0.25 ± 0.05
	Depth	−0.30 ± 0.08	−0.29 ± 0.07	−0.29 ± 0.07	−0.32 ± 0.07	−0.2 ± 0.04	−0.31 ± 0.06
MDH	Intercept	5.28 ± 0.34	4.95 ± 0.32	4.98 ± 0.31	4.94 ± 0.31	4.31 ± 0.25	4.73 ± 0.3
	Loc. mode	−0.14 ± 0.12	−0.19 ± 0.12	−0.16 ± 0.12	−0.19 ± 0.12	−0.24 ± 0.12	−0.18 ± 0.12
	Mass	−0.07 ± 0.04	−0.07 ± 0.04	−0.08 ± 0.04	−0.07 ± 0.04	−0.10 ± 0.04	−0.10 ± 0.04
	Depth	−0.32 ± 0.05	−0.27 ± 0.05	−0.28 ± 0.05	−0.28 ± 0.05	−0.17 ± 0.03	−0.23 ± 0.05
LDH	Intercept	7.72 ± 0.52	7.15 ± 0.49	7.15 ± 0.48	7.16 ± 0.47	5.95 ± 0.41	6.89 ± 0.45
	Loc. mode	0.44 ± 0.17	0.34 ± 0.18	0.40 ± 0.18	0.35 ± 0.18	0.28 ± 0.19	0.36 ± 0.18
	Mass	0.04 ± 0.06	0.05 ± 0.07	0.02 ± 0.07	0.04 ± 0.07	−0.03 ± 0.07	−0.01 ± 0.06
	Depth	−0.54 ± 0.08	−0.47 ± 0.08	−0.47 ± 0.08	−0.48 ± 0.08	−0.26 ± 0.05	−0.43 ± 0.07
PK	Intercept	6.3 ± 0.44	5.92 ± 0.4	5.88 ± 0.4	5.9 ± 0.39	4.98 ± 0.33	5.63 ± 0.38
	Loc. mode	0.26 ± 0.15	0.19 ± 0.15	0.24 ± 0.15	0.19 ± 0.15	0.14 ± 0.16	0.20 ± 0.15
	Mass	−0.01 ± 0.05	0.00 ± 0.06	−0.03 ± 0.05	−0.01 ± 0.06	−0.06 ± 0.06	−0.05 ± 0.05
	Depth	−0.41 ± 0.07	−0.37 ± 0.06	−0.36 ± 0.06	−0.37 ± 0.06	−0.21 ± 0.04	−0.32 ± 0.06

Table 4
Generalized linear model results for enzyme activities of select particular phylogenetic groups. Models are fit to maximum, median and minimum depth estimates for species using empirical trawl data from the present study and from the literature. Shading indicates significance (light grey – $p < 0.05$, dark grey – $p < 0.01$, black – $p < 0.001$). Unshaded cells were not significant while cells marked with an x indicate no test.



activities compared to the influence of median depth of occurrence. Only glycolytic activities declined with depth in elasmobranchs possibly due to differences in lactate processing physiology compared to teleosts (Condon et al., 2012).

While the decline in metabolic enzyme activities with depth seen in this study for benthopelagic species is similar to previous examinations (Seibel and Drazen, 2007), the patterns for benthic species are new. The visual interactions hypothesis explains declines in metabolic rates with depth through the reduction in selection for robust locomotion for predator–prey interactions due to declining light levels. Initially based on data for demersal crustaceans, it was hypothesized that benthic species, which have opportunities for camouflage and burrowing at all depths, would not show metabolic declines with depth aside from those due to temperature (Childress et al., 1990). This has been confirmed for benthic octopus (Seibel and Childress, 2000), hagfish (Drazen et al., 2011), crustaceans and echinoderms (Hughes et al., 2011; Wilson et al., 2013). In contrast, our results suggest lower potential metabolic rates for benthic fishes in deeper waters.

Unlike invertebrates in earlier studies, benthic fishes still swim. Even benthic species which are sedentary and well-camouflaged will have escape or predation responses which may require greater metabolic capacity in well-lit shallow water environments. The same explanation has been used for benthic caridean shrimps which spend more time off the seafloor than crabs or other shrimp taxa and exhibit modest declines in metabolic rate with depth (Childress et al., 1990; Seibel and Drazen, 2007). Additionally, the higher enzymatic activities exhibited in shallow-living benthic fish may be reflective of the greater locomotory capabilities required to maintain or change position in highly turbulent environments. This has been seen at smaller depth scales in benthic triplefin fishes where enzymatic activity in the trunk musculature correlates to the water velocity of their intertidal to subtidal habitats (Hickey and Clements, 2003). Although some benthic fish can burrow, they may be subject to disruptive turbulence that

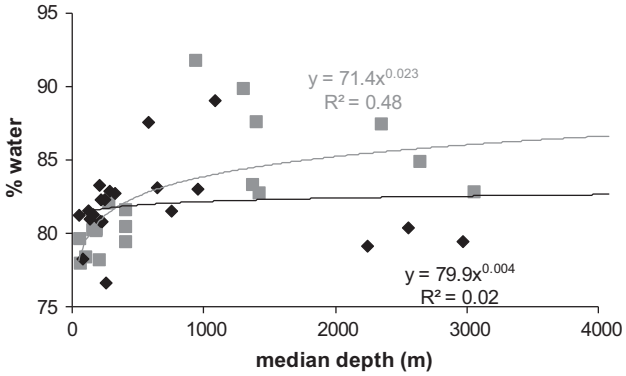


Fig. 2. Water content of white muscle tissue of benthic (black diamonds) and benthopelagic (gray squares) species as a function of median depth of occurrence. Data are from the present study and from Drazen (2007).

requires greater locomotory and hence enzymatic capacity to hold station on the seafloor or to swim to a new location. Current velocities and turbulent flow generally decline with increasing depth (Stabeno and Smith, 1987), though these patterns can be strongly affected by local bathymetry (e.g. Wilson and Boehlert, 2004). Thus benthic fishes, unlike echinoderms or crabs, may still experience relaxed selective pressure for high capacity locomotion with depth. Reductions in red muscle proportions and enzymatic activity were seen in benthic fishes suggesting reductions in routine locomotory ability, likely for similar reasons (Drazen et al., 2013).

As expected, CS activity was significantly different between the two locomotory modes. Our assays were conducted on white muscle which is predominantly used for burst locomotory activity; but some fishes can use white muscle fibers for intermediate levels of routine swimming activity (Johnston and Moon, 1981; Sanger and Stoiber, 2001). This may be the reason for the higher CS values in benthopelagic fishes which are found in the water column as opposed to resting on the seafloor. Ombres et al. (2011) found that schooling species of rockfish had higher white muscle CS activities compared to species living closer to the bottom as ambush predators. There is similar evidence of variation with *Sebastes* spp. in our study too. For instance *Sebastes goodei* and *Sebastes jordani* are generally active schooling species (Lenarz, 1980) and had high CS values whereas more sedentary species such as *Sebastes melanostoma* and *Sebastes aurora* have lower activities (Table 1) though this result also covaries with depth. Furthermore, in situ data also suggest that benthopelagic species tend to have higher metabolic rates than benthic species (Drazen and Yeh, 2012). This is also indicated by their higher proportions of red muscle (Drazen et al., 2013). At greater depths, the glycolytic enzyme activities are slightly higher in benthic species, although this was only significant in the case of LDH. These differences are small but they could be due to more robust "sit and wait" predation strategies associated with the need for greater burst locomotory capacity.

The Zoarcids are an exception where our broad dichotomous characterization of locomotory mode probably does not equate to metabolic and locomotory activity levels. The two benthopelagic species had lower CS than benthic species and lower glycolytic enzyme activities (Table 3). These two species were classified as benthopelagic because *Bothrocara molle* has been captured tens of meters above the seafloor (Anderson, et al., 2009) and *Bothrocara bruneum* is seen hovering above bottom (Ferry, 1997) and has been captured above the bottom by ROV (Drazen and Robison, unpub data). Furthermore, *B. bruneum* eats mainly pelagic/benthopelagic crustaceans and fish, a diet unlike other zoarcids off California (Ferry, 1997). Both of these species have high white muscle water content, 87.7 and 87.5% respectively compared to an average of 81.0% for 5 benthic zoarcids. This difference is not large enough to explain the difference in enzyme activities directly: the activities expressed per gram dry weight produced the same trends (data not shown). However, high water content is likely an adaptation to achieve neutral buoyancy for a benthopelagic lifestyle without a gasbladder (Drazen, 2007). The Alepocephalids in this study also have high water content, lack a gasbladder and have low enzymatic activities indicative of low metabolic and locomotory abilities (Table 1). These fishes may adopt a principally float or drift lifestyle which has been observed for *B. brunneum* (Ferry, 1997) and several Alepocephalids (Lorance and Trenkel, 2006). This behavior would allow for watery muscle, and does not often necessitate high activity. These examples point out that there is a great deal of complexity in fish swimming and activity that is not captured by the broad dichotomy of benthic vs. benthopelagic but that broad differences are discernible using metabolic enzyme activities.

Four taxa permitted more detailed analysis of depth patterns and these generally followed those for the larger locomotory mode groups. In the case of the benthopelagic Gadiformes, species analyzed were virtually all deep-living so no patterns were evident as most of the decline in enzyme activities across all species appears to occur in the first several hundred meters water depth (Fig. 1). The genus *Sebastes* had significant declines in all but CS activities which, for a benthopelagic genus, was suggestive of reductions in locomotory performance as predicted by the visual interactions hypothesis. Declines in these four enzyme activities were also found in earlier work which combined two *Sebastes* spp. with the two *Sebastolobus* spp. (Vetter and Lynn, 1997). The two benthic taxa, Pleuronectiformes and Zoarcidae, both showed depth-related declines in most enzymes and for some and nearly all depth scenarios, respectively, similar to the overall pattern for benthic species. Earlier investigations have shown that the deepest flatfish species, *Microstomus pacificus* and *Embassichthys bathybius*, have gelatinous muscle (Drazen, 2007; Hunter et al., 1990) and as adults are adapted to live in low oxygen conditions (Friedman et al., 2012; Hunter et al., 1990; Vetter et al., 1994). The oxygen minimum zone (OMZ) occurs from roughly 600 to 1000 m off the coast of Monterey Bay (Friedman et al., 2012). For our other groups there are data for species below the OMZ and they don't show an increase in metabolic enzyme activities suggesting that the OMZ is not a major determinant of enzymatic activities interspecifically. Indeed in benthic fishes a recent study shows that they are adapted mainly by increasing gill surface area, thereby increasing their ability to extract oxygen in low concentration environments (Friedman et al., 2012). The patterns for individual taxa are best explained by the reasons given above for benthic species overall.

This study represents the first comprehensive examination of metabolic enzyme activities of demersal fish species across a 3000 m depth range. The results confirm early less comprehensive analyses showing that enzyme activities decline with depth in benthopelagic fishes. Although, previous work was not able to reach firm conclusions about the patterns for benthic species, this study shows that their enzyme activities also decline with depth. This indicates that shared environmental factors may be influencing swimming capability, regardless of locomotory mode within the water column, resulting in higher metabolic and locomotory capacities in shallow water for all species. Metabolic rate does not vary simply as a function of mass and temperature in fishes as shown by the substantial depth-related changes in enzymatic activities. Together with depth-related declines in growth rates and productivity (Drazen and Haedrich, 2012), the present results confirm that the pace of life in deep-sea fishes is much lower than those in shallow water. Vital rates of metabolism and growth could be severely overestimated by models based solely on mass and temperature which could lead to underestimates of deep-sea fishes' susceptibility to anthropogenic stresses.

Acknowledgments

We thank Shaara Ainsley, Mariah Boyle, Donna Kline, Carrie Laxson, Jackie Lighten, Katie Schmidt, Paul Yancey, and John Yeh who participated in the sampling cruises. In addition to her support in the Field, Michelle Kay provided educational outreach through her website http://deepblusea2009.blogspot.com/. Dana Sackett assisted with the GLM analysis. Thanks to the captain and crew of the R/V Point Sur for two fun and productive cruises. This research was conducted in accordance with University of Hawaii Institutional Animal Care and Use Committee protocols. NSF (OCE0727135) and NOAA-NWFSC provided funding for this work. This is SOEST contribution 9265.

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