Journal of FISH BIOLOGY

fsbi An International Society for Fish Biology

Journal of Fish Biology (2013) **83**, 1592–1612
doi:10.1111/jfb.12268, available online at wileyonlinelibrary.com

Red muscle proportions and enzyme activities in deep-sea demersal fishes

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Owing to the paucity of data on the red muscle of deep-sea fishes, the goal of this study was to determine the proportions of red muscle in demersal fishes and its enzymatic activities to characterize how routine swimming abilities change with depths of occurrence. Cross sectional analysis of the trunk musculature was used to evaluate the proportion of red muscle in 38 species of Californian demersal fishes living at depths between 100 and 3000 m. The activity of metabolic enzymes was also assayed in a sub-set of 18 species. Benthic fishes had lower proportions of red muscle and lower metabolic enzyme activities than benthopelagic species. Mean proportion of red muscle declined significantly with depth with the greatest range of values in shallow waters and species with low proportions found at all depths. This suggested that while sedentary species occur at all depths, the most active species occur in shallow waters. Citrate synthase activity declined significantly with depth across all species, indicating that the mass-specific metabolic capacity of red muscle is lower in deep-sea species. These patterns may be explained by coupling of red and white muscle physiologies, a decrease in physical energy of the environment with depth or by the prevalence of anguilliform body forms and swimming modes in deep-living species.

Key words: citrate synthase; locomotion; metabolic adaptation; Monterey Bay; visual interactions hypothesis.

INTRODUCTION

Metabolic and biochemical adaptations of deep-sea fishes have been well studied. In general, the metabolic capacities of deep-living species are lower than shallow-living ones. Experiments on whole animals have shown reduced metabolic rates (up to an order of magnitude) in pelagic and benthopelagic teleosts (Torres *et al.*, 1979; Bailey *et al.*, 2002; Drazen & Yeh, 2012). Such experiments are difficult to conduct and data are limited. The activities of several key metabolic enzymes have proven to be useful proxies for metabolic capacity across different fish taxa (Childress & Somero, 1979; Dalhoff, 2004; Drazen & Seibel, 2007). A number of enzymes have been utilized but most often the glycolytic enzymes, pyruvate kinase (PK) and lactate dehydrogenase (LDH), and the tricarboxylic acid (TCA) cycle enzymes, malate dehydrogenase (MDH) and citrate synthase (CS), are assayed because they generate ATP for muscle contraction and have correlated with whole-body metabolic rate (Childress & Somero, 1979; Torres & Somero, 1988). Activities of these enzymes

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in white muscle (but not in brain or heart) decline with depth of occurrence in deep-sea teleosts (Childress & Somero, 1979; Sullivan & Somero, 1980; Drazen & Seibel, 2007) and elasmobranchs (Treberg *et al.*, 2003; Condon *et al.*, 2012) generally corroborating respirometry measurements. It is thought that metabolism declines because increasing darkness reduces the distances over which predators and prey react to each other thus relaxing the selective pressure for high-speed locomotory abilities and associated metabolic capacity (Seibel & Drazen, 2007). The teleost studies are based on the analysis of white muscle tissue.

Fish muscle can be classified into red, white and intermediate or pink fibres (Altringham & Ellerby, 1999; Sanger & Stoiber, 2001). Red and white muscles make up most of the fibres and they are different not only in colour, but also in composition, placement or situation in the body, histology and size. Red muscle is typically only a small proportion of the total muscle mass and is generally found beneath the lateral line and near the horizontal septum. Relative to white muscle, red muscle fibres are narrow, have high concentrations of mitochondria, haemoglobin (found in the blood) and myoglobin (Greer-Walker & Pull, 1975; Sanger & Stoiber, 2001) and high activities of TCA enzymes such as MDH and CS (Johnston & Moon, 1981). A higher amount of myoglobin and greater vascular supply in red muscle is the cause for the colour disparity between the two types of muscles and indicates its aerobic poise (Sanger & Stoiber, 2001). The red muscle tissue is used primarily for continuous directional locomotion or station holding in the face of slow currents. Conversely, white muscle fibres are larger in diameter, have much lower vascular supply and high levels of glycolytic enzymes and they are primarily responsible for high-speed or burst locomotory behaviours such as those found in predator–prey interactions (Mosse & Hudson, 1977; Somero & Childress, 1980; Altringham & Ellerby, 1999). In addition, some fishes may use white muscle fibres to a certain extent for intermediate levels of swimming activity (Johnston & Moon, 1981; Sanger & Stoiber, 2001). A study comparing red muscle enzyme activities between one shallow and one deep-living teleost found that the deeper-living species had significantly lower activities (Yang & Somero, 1993). Without results for more species, the generality of this finding cannot be ascertained.

The proportion of red muscle in cross section of the trunk of fishes (relative to total muscle area) has been shown to relate to sustained swimming activity (Greer-Walker & Pull, 1975; Mosse & Hudson, 1977; Hickey & Clements, 2003; Cediel *et al.*, 2008). More active species have proportionally more red muscle than sedentary ones, although there is a continuum of ratios rather than the convenient dichotomy between sedentary and mobile species (McLaughlin & Kramer, 1991). These studies have compared relative amounts of red muscle in shallow-living marine and freshwater species but little has been done on deep-sea fishes. Blaxter *et al.* (1971) found a large range (0–15%) in a small number of meso and bathypelagic species. Greer-Walker & Pull (1975) performed a comprehensive survey of 84 species including a few demersal deep-sea species finding proportions of $<10\%$ which in comparison with shallow-living fishes was interpreted as suggesting a 'sluggish character'. Direct measurements of locomotion in a few deep-sea fishes suggest that at least some have comparable routine swimming velocities to shallow-living fishes at similar temperatures, although the specimens had been stimulated by bait to attract them to the experiment (Collins *et al.*, 1999; Bailey *et al.*, 2005*a*).

Owing to the paucity of data on the red muscle of deep-sea fishes, the goal of this study was to determine the proportion of red muscle in demersal fishes and its enzymatic activities. This information will help characterize how the routine swimming abilities of fishes change across their depths of occurrence. On the basis of the previous work, it was hypothesized that the proportion of red muscle and its enzymatic activities should decline with depth.

MATERIALS AND METHODS

Demersal fishes were collected by trawl during two oceanographic research expeditions in 2009 off Monterey Bay, CA, U.S.A. (36° 46' N; 122° 00' W). Trawls were conducted between *c*. 100 and 3000 m. The mass and standard length (pre-anal fin length for macrourids) of the fishes were measured. The depths of occurrence of each species were taken from this sampling and also from the literature. Deep-sea fishes represent a variety of locomotory modes and lifestyles in the benthic environment. They have been very broadly classified as either benthic or benthopelagic (Drazen & Seibel, 2007) with the latter term referring to generally more mobile species which spend most of their time swimming in the water column rather than resting on the seafloor. This classification is rather anecdotal but in the absence of any other information it is employed cautiously here.

ENZYMATIC ASSAYS

Red muscle was taken from near the lateral line in the posterior third of each fish and frozen in liquid nitrogen. All tissues were stored at −80° C until preparation of the homogenates. Tissue samples of *c*. 0·1 g were homogenized with the addition of 1 ml of ice-cold 10 mM tris–HCl buffer (pH 7·8 at 10° C).

Four enzymes were assayed for their maximum activities using the same methods as for past studies of white muscle tissue. CS catalyses the first step in the TCA cycle, serving an important role in oxidative metabolism (Somero & Childress, 1980) and correlates with mitochondrial density (Moyes *et al.*, 1992). MDH is not only a part of the TCA cycle but also serves to maintain redox balance between the mitochondria and cytoplasm (Siebenaller *et al.*, 1982; Gelpi *et al.*, 1992; Ombres *et al.*, 2011). LDH is the final enzyme of anaerobic glycolysis producing lactate. Thus, its activity is indicative of anaerobic capacity and, in white muscle, of burst locomotory capacity interspecifically (Childress & Somero, 1979; Dalhoff, 2004). PK catalyses 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 *v*. aerobic metabolism suggests that activities of PK are more likely to indicate anaerobic potential of the tissue. Other studies do find positive correlations between PK and LDH in white muscle (Childress & Somero, 1979; Sullivan & Somero, 1980; Ombres *et al.*, 2011). All assays followed the procedures detailed in the study by Condon *et al.* (2012). The amount of homogenate used in the assays was varied in order to ensure that concentrations of metabolites were not limiting. Measurements were made in a spectrophotometer externally cooled to 10° C. Activity levels are expressed as units (μmoles product g wet mass^{-1} min^{-1}).

PROPORTIONS OF RED MUSCLE

Fishes from trawls were cut into five sections starting at the first dorsal ray and ending at the caudal peduncle. The first section was directly after the first dorsal ray, the second was at the anus and the rest of the body was divided into sections of equal width (Fig. 1). Ideally, cross sections are analysed histologically to determine the fibre types and distributions (Zhang *et al.*, 1996; Cediel *et al.*, 2008). This is difficult, however, when large fishes such as those in this study are examined. In addition, here, the question concerned interspecific differences in the proportion of aerobic (mostly red) fibres rather than a detailed characterization of fibre

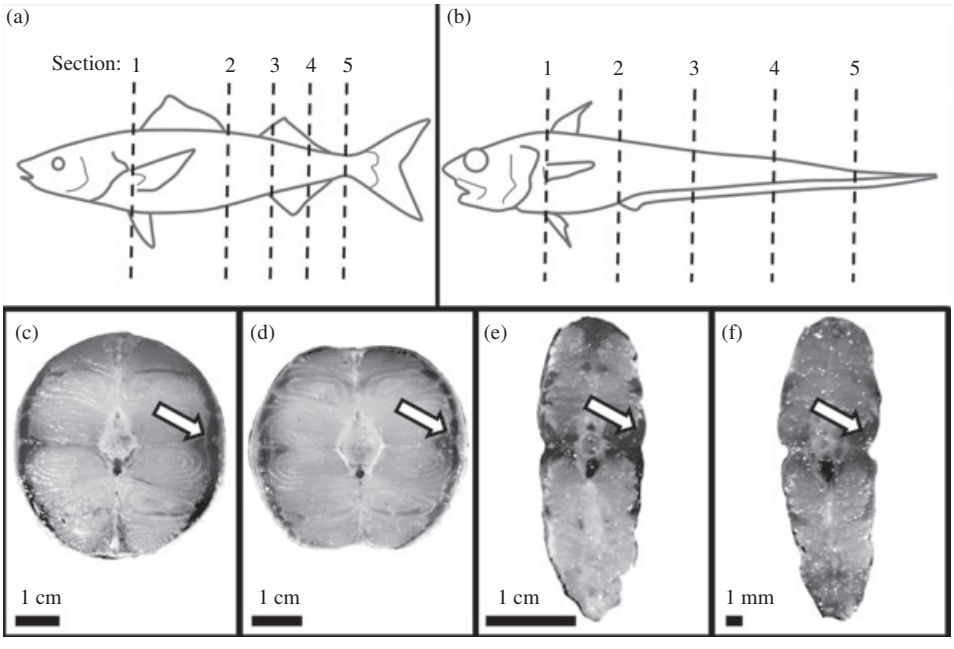

FIG. 1. Relative locations for cross sectioning and sample images from fishes of two different body morphologies: (a) *Anoplopoma fimbria* and (b) the anguilliform *Coryphaenoides acrolepis*. (c, d) Representative photographs used for analysis of *A. fimbria*, section numbers 4 and 5. (e, f) Representative photographs used for analysis of *C. acrolepis*, section numbers 4 and 5. ⇨, the boundary of red (darker) and white (lighter) muscle.

types. As such, an approach similar to others with similar questions was taken (Greer-Walker & Pull, 1975; Johnston *et al.*, 2003) using macroscopic analysis of photographs (Fig. 1). For the determination of red and white muscle areas, photographs were analysed using image analysis software (Adobe Photoshop CS; www.adobe.com). The areas of red and white muscle were traced using a Wacom digital tablet (www.wacom.com). Red and white muscles were distinguished on the basis of colour. One half of the cross section was used for analysis because of the bilaterally symmetrical musculature of teleosts (Zhang *et al.*, 1996; Cediel *et al.*, 2008). For calculation of the percentage of red muscle, the area of red muscle was divided by the total muscle area of the section (Zhang *et al.*, 1996; Cediel *et al.*, 2008). Each cross section was examined thrice and the average of these values was used in data analysis and cross species comparison.

In a few specimens, all cross sections were analysed and it was found that the greatest proportions of red muscle were in the posterior sections, similar to findings from past studies (Zhang *et al.*, 1996; Altringham & Ellerby, 1999). Thus, for the remaining specimens, the two most posterior sections were examined. It is data from these two locations that were used to make comparisons among species. The use of paired fins for locomotion has been shown to reduce the amount of red muscle in the trunk musculature (Greer-Walker & Pull, 1975; Rosenblatt & Johnson, 1976). All of the fishes examined have been observed to swim *via* undulations of body musculature rather than using paired fins (J. C. Drazen, J. P. Barry & B. H. Robison, unpubl. obs.).

Most species were from deeper waters so literature values were incorporated for marine demersal teleosts which are not pectoral swimmers (Greer-Walker & Pull, 1975; Mosse & Hudson, 1977). These data have been reviewed in the study by McLaughlin & Kramer (1991) and body location corresponds to section 5 shown in Fig. 1 (Fig. 1). Minimum and maximum depths typically inhabited for each species as adults and whether each species was benthic or

benthopelagic was ascertained from the literature where possible, as in past studies (Drazen & Haedrich, 2012; Appendix). The percentage data were analysed using analysis of covariance (ANCOVA) with lifestyle (benthic or benthopelagic) and depth as explanatory variables. All percentages were logit transformed prior to analysis and tested for normality. In addition, a Breusch–Pagan test was employed to evaluate homogeneity of variances as a function of depth (Breusch & Pagan, 1979).

RESULTS

MUSCLE ENZYMATIC ACTIVITIES

The enzymatic activities of red muscle were measured for 18 species of fish (Table I). Most of these species were benthopelagic. The highest CS activity values were found in shallow benthopelagic species, chilipepper rockfish *Sebastes goodei* (Eigenmann & Eigenmann 1890) and white croaker *Genyonemus lineatus* (Ayres 1855), and the lowest value was found for a deep-living benthic species, shortspine thornyhead *Sebastolobus alascanus* Bean 1890. *Sebastolobus alascanus* also had the lowest MDH value while the highest was in the deep-living pudgy cuskeel *Spectrunculus grandis* (Günther 1877) and activities of this enzyme ranged widely amongst the species. For the glycolytic enzymes, LDH activities were always higher than PK and the lowest of both were found in the California slickhead *Alepocephalus tenobrosus* Gilbert 1892. The Pacific rattail *Coryphaenoides acrolepis* (Bean 1884) had the highest LDH and both sablefish *Anoplopoma fimbria* (Pallas 1814) and *G. lineatus* had the highest PK activities. Although the focus of the study was to survey across many species, few had sufficient replicates (five or more) to adequately evaluate the effects of mass on enzymatic activity. In *C. acrolepis*, both CS (y; units g^{-1}) and MDH (z; units g^{-1}) scaled negatively with body mass (M; g) [$y = 656\,M^{-0{\cdot}526}$ ($r^2 = 0{\cdot}84$, $P < 0{\cdot}05$); $z = 197\,640\,M^{-0{\cdot}917}$ ($r^2 = 0{\cdot}80$, $P < 0{\cdot}05$)].

Depth and basic locomotory mode did affect the enzymatic activities to some degree. Only MDH activities differed significantly between the benthic and benthopelagic species (Mann–Whitney U-test: $U_{3,15} = 41$, $P > 0{\cdot}05$; Table I). The three benthic species, however, did have some of the lowest CS activities (Mann–Whitney U-test: $U_{3,15} = 3$, $P > 0{\cdot}05$). Mean values of LDH and PK were higher for benthopelagic species compared to benthic ones but interspecific variability was very high and the differences were not significant. CS activities declined exponentially and significantly with depth ($r^2 = 0{\cdot}35$, $P < 0{\cdot}05$; Fig. 2). Depth did not correlate significantly to the other enzyme activities. Regressions to either maximum depth or minimum depth resulted in minor changes to the slopes and intercepts. Removing the benthic species ($n = 3$) from the regression also had little influence except to increase the variance explained in CS ($r^2 = 0{\cdot}48$, $P < 0{\cdot}01$; Fig. 2). No regressions were performed for the three benthic species but the CS activity of the shallower-living petrale sole *Eopsetta jordani* (Lockington 1879) was much higher than the two deeper-dwelling *Sebastolobus* species (Table I).

PROPORTIONS OF RED MUSCLE

Many fishes in the deep sea are eel-like and lack a large caudal fin (Marshall, 1954). Most studies of the distribution of red muscle along the body have been

TABLE I. Red muscle enzymatic activities (U g^{-1} wet mass) at 10° C (mean ± S.D.). Lifestyle is either benthic (B) or benthopelagic (BP)

Species	Mass range (g)	n	Lifestyle	CS	MDH	LDH	PK
Albatrossia pectoralis	1000–4000	6	BP	9·8 ± 2·7	115·2 ± 39·7	85·9 ± 47·3	40·9 ± 21·7
Alepocephalus tenebrosus	1579–2480	2	BP	9·8 ± 1·4	283 ± 13·3	32·7 ± 7·0	14·7 ± 3·2
Anoplopoma fimbria	205–3825	11	BP	16·5 ± 4·6	139·5 ± 36·7	142·6 ± 33·3	66·1 ± 14·8
Antimora microlepis	336–1693	6	BP	18·0 ± 3·3	183·8 ± 35·5	87·6 ± 24·7	44·9 ± 6·0
Coryphaenoides acrolepis	743–2195	5	BP	14·6 ± 3·9	272·6 ± 153·0	224·2 ± 81·3*	33·4 ± 14·4
Coryphaenoides armatus	672–1499	5	BP	15·9 ± 8·4	377·8 ± 92·7	153·9 ± 36·0	53·5 ± 50·8
Coryphaenoides filifer	485–1103	4	BP	8·6 ± 1·6	284·7 ± 45·1	159·3 ± 40·9	18·6 ± 7·0
Eopsetta jordani	631–1071	2	B	16·5 ± 2·5	128·5 ± 5·7	55·7 ± 6·6	38·8 ± 1·8
Genyonemus lineatus	113–179	3	BP	31·6 ± 3·7	316·5 ± 117	158·0 ± 27·2	62·0 ± 18·2
Merluccius productus	569–673	3	BP	17·8 ± 1·2	174·6 ± 49·4	115·9 ± 7·3	32·7 ± 4·1
Sebastes crameri	393	1	BP	22·1	330·8	173·2	101·9
Sebastes diploproa	278	1	BP	9·5	96·2	134·4	19·8
Sebastes goodei	310–1154	4	BP	40·1 ± 15·7	255·8 ± 37·6	234·4 ± 117·0	208·2 ± 99·3
Sebastes jordani	122	1	BP	22·2	293·9	171·4	63·7
Sebastes melanostomas	646	1	BP	21·2	190·0	191·2	188·7
Sebastolobus alascanus	2265–3565	2	B	6·2 ± 1·8	89·6 ± 10·5	92·1 ± 5·7	28·7 ± 3·2
Sebastolobus altivelis	192	1	B	7·5	127·1	180·9	16·4
Spectrunculus grandis	607–17 463	3	BP	11·0 ± 0·6	304·2 ± 25·8	nd	52·2 ± 5·3
All benthic species		3		10·1 ± 5·6	115·0 ± 22·1	110·0 ± 64·4	28·0 ± 11·2
All benthopelagic species		15		17·9 ± 8·8	241·0 ± 85·2	148·0 ± 54·5	66·7 ± 58·1

n, sample size; nd, no data; CS, citrate synthase; MDH, malate dehydrogenase; LDH, lactate dehydrogenase; PK, pyruvate kinase *$n = 3$.

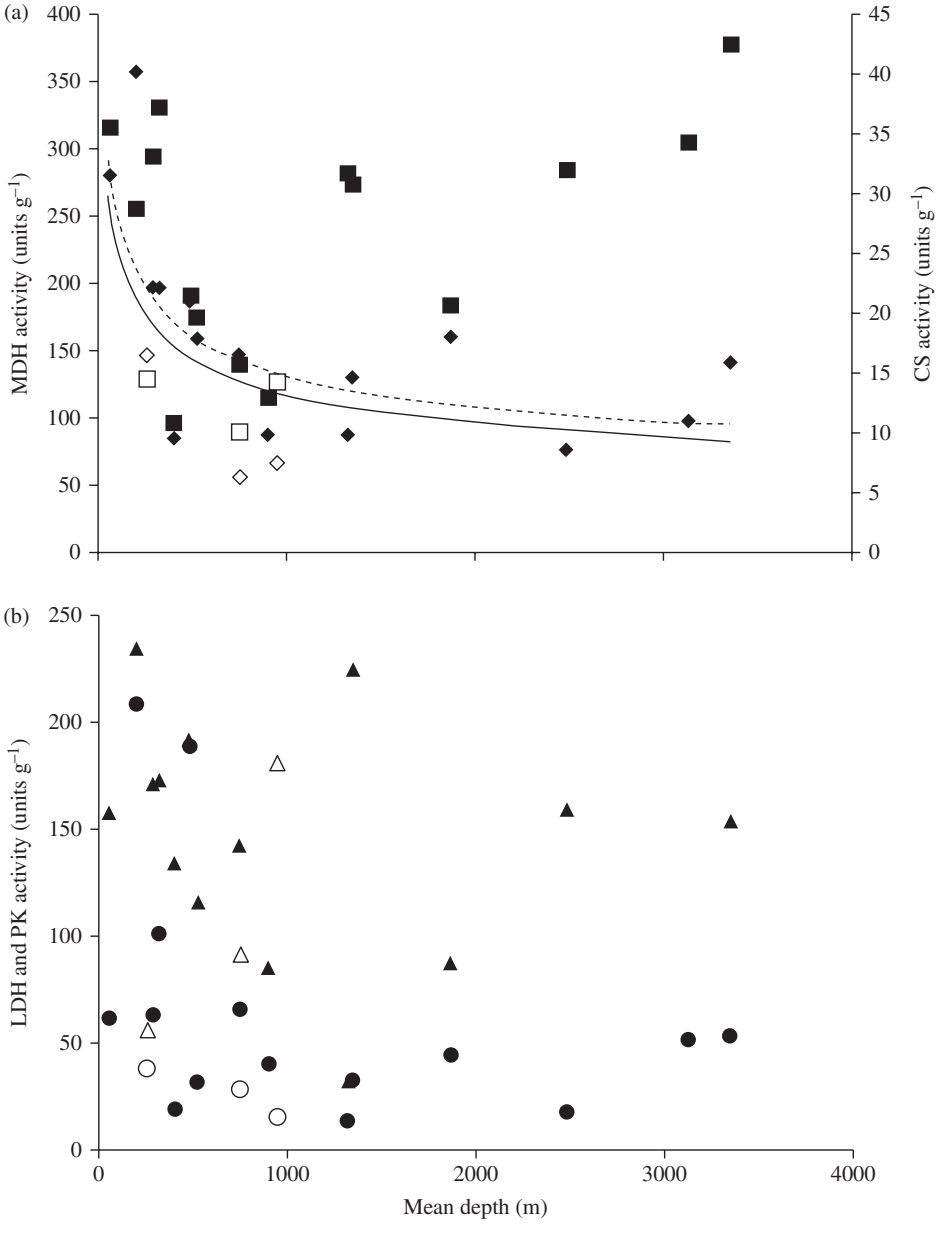

FIG. 2. (a) Aerobic and (b) glycolytic enzyme activities of demersal fishes as a function of minimum depth of occurrence. Benthic species are represented by open symbols. CS, citrate synthase (◆); MDH, malate dehydrogenase (■); LDH, lactate dehydrogenase (▲); PK, pyruvate kinase (●). (a) The curves were fitted by $y = 91{\cdot}3x^{-0{\cdot}28}$ ($r^2 = 0{\cdot}35$) (___) and without the benthic species included $y = 99{\cdot}5x^{-0{\cdot}28}$ ($r^2 = 0{\cdot}48$) (------).

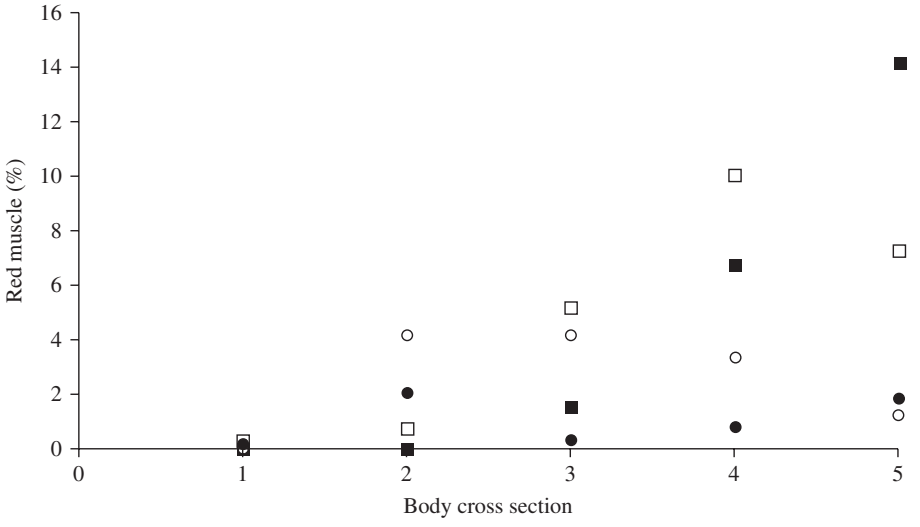

FIG. 3. Red muscle as a percentage of the total muscle area in cross section at points along the body of the fish from anterior (1) to posterior (5). Points are for individual fishes: *Coryphaenoides armatus* (□, ■), *Pachycara bulbiceps* (●) and *Pachycara gymninium* (○).

conducted on species with well-defined caudal fins (Greer-Walker & Pull, 1975). Thus, four fish specimens with eel-like tails were examined for red muscle at all five points along the body from just after the first dorsal fin to the tail (Fig. 3). For both specimens of the abyssal grenadier *Coryphaenoides armatus* (Hector 1875), proportions of red muscle increased along the length and were highest posteriorly as has been shown in most species (Zhang *et al.*, 1996). Much less red muscle was present in two eelpouts, nakedneck eelpout *Pachycara gymninium* Anderson & Peden 1988 and Snubnose eelpout *Pachycara bulbiceps* (Garman 1899) with a more homogenous distribution along the body. *Pachycara gymninium* did show a peak in red muscle in the medial body sections numbered 2 and 3, with a moderate reduction at section 4 (Fig. 1). On the basis of these results and previous research (Zhang *et al.*, 1996; Sanger & Stoiber, 2001, it was decided to use sections 4 and 5 to characterize the proportion of red muscle in each individual.

A total of 38 species were evaluated (Table II) and the percentage of red muscle varied from 0 to 13·5% at section 4 and from 0 to 18·7% at section 5. Variability between individuals was high for some species but interspecific variability was much higher. On average, section 5 had more red muscle than section 4 when comparing within individual specimens (paired t-test, $t = 5{\cdot}36$, d.f. $= 37$, $P < 0{\cdot}001$). Benthic species had lower proportions of red muscle than benthopelagics in either section [ANCOVA, $F_{1,34} = 13{\cdot}6$ (section 4), $F_{1,34} = 18{\cdot}4$ (section 5), $P < 0{\cdot}001$; Table II]. Within the benthic species, the *Sebastolobus* spp. had the highest percentage of red muscle (9–11·5%) and the rest of the species ranged from 0 to 4·5%. The range was much higher for benthopelagic species with the benthopelagic two line eelpout *Bothrocara brunneum* (Bean 1890) and the ghostly grenadier *Coryphaenoides leptolepis* Günther 1877 having the lowest values and the giant grenadier *Albatrossia pectoralis* (Gilbert 1892) and *C. acrolepis* having the highest values.

TABLE II. Red muscle as percentage of total muscle area (mean ± S.D.) at two locations along the body of the fishes (see Fig. 1)

Species	*n*	Lifestyle	Mass range (g)	Section 4	Section 5
Albatrossia pectoralis	2	BP	896·8–1568·9	13·5 ± 4·7	18·5 ± 6·8
Alepocephalus tenebrosus	2	BP	589·0–1578·9	8·2 ± 0·1	10·3 ± 0·5
Anoplopoma fimbria	4	BP	1217·0–1989·2	10·0 ± 0·7	14·1 ± 5·4
Antimora microlepis	2	BP	549·2–775·9	11·1 ± 2·7	15·4 ± 0·7
Asterotheca pentacantha	1	B	13·9	0·0	0·2
Atheresthes stomias	1	B	371·4	3·8	3·2
Bothrocara brunneum	3	BP	606·5–890·1	1·5 ± 0·6	2·7 ± 1·4
Chilara taylori	3	B	16·1–69·5	1·2 ± 1·2	2·5 ± 0·4
Citharichthys sordidus	2	B	37·9–81·0	3·4 ± 1·8	4·5 ± 0·7
Coryphaenoides acrolepis	3	BP	699·2–1267·2	11·7 ± 2·0	18·7 ± 5·7
Coryphaenoides armatus	6	BP	66·1–1102·1	6·5 ± 2·0	8·2 ± 3·6
Coryphaenoides filifer	4	BP	356·5–356·5	12·7 ± 1·9	13·9 ± 1·3
Coryphaenoides leptolepis	2	BP	85·2–85·2	2·0 ± 1·5	3·8 ± 2·2
Coryphaenoides yaquinae	1	BP	189·4	6·4	7·2
Embassichthys bathybuis	1	B	295·5	1·6	1·6
Errex zachirus	1	B	121·8	1·0	0·7
Genyonemus lineatus	2	BP	169·2–223·2	10·5 ± 2·9	13·8 ± 2·3
Icelinus tenuis	2	B	12·0–16·1	1·0 ± 1·5	0·5 ± 0·7
Lycenchelys sp.	3	B	87·5–112·1	1·2 ± 1·5	0·8 ± 1·3
Lycodes cortezianus	1	B	58·3	2·1	1·7
Lycodes diapterus	2	B	47·6–81·2	0·0 ± 0·0	0·0 ± 0·0
Nezumia liolepis	1	BP	54·4	4·9	5·7
Ophiodon elongatus	2	B	50·2–65·5	1·2 ± 0·8	1·7 ± 0·1
Pachycara bulbiceps	1	B	503·6	0·6	3·4
Pachycara gymnium	1	B	900·9	3·2	1·9
Pachycara lepinium	1	B	490·7	0·0	0·0
Parophyrs vetulus	1	B	66·7	0·8	1·4
Sebastes aurora	1	BP	311·6	7·0	10·2
Sebastes diploproa	1	BP	37·0	6·5	11·1
Sebastes elongatus	2	BP	29·5–95·4	3·2 ± 1·5	5·8 ± 4·7
Sebastes goodei	1	BP	387·8	8·9	13·0
Sebastes jordani	1	BP	121·5	7·2	11·2
Sebastes melanostomus	2	BP	525·0–747·0	7·6 ± 5·2	8·2 ± 5·0
Sebastes proriger	1	BP	201·1	9·5	10·8
Sebastes saxicola	2	BP	25·5–95·8	3·6 ± 4·3	4·2 ± 0·6
Sebastolobus alascanus	3	B	232·0–2480·1	8·7 ± 0·5	11·5 ± 6·8
Sebastolobus altivelis	4	B	111·5–309·0	5·4 ± 1·7	90·0 ± 1·8
Spectrunculus grandis	1	BP	606·5	7·4	7·4
All benthic species	17			2·1 ± 2·3	2·6 ± 3·2
All benthopelagic species	21			7·6 ± 3·4	10·2 ± 4·6

n, sample size; B, benthic; BP, benthopelagic.

There was no significant relationship between depth and the proportion of red muscle in either section 4 or 5 or for benthic or benthopelagic groups [ANCOVA, $F_{1,34} = 0{\cdot}48$ (section 4), $F_{1,34} = 0{\cdot}21$ (section 5), $P > 0{\cdot}05$ for depth in all cases and $P > 0{\cdot}05$ for the interaction between groups and depth]. When literature values (Appendix) were added, however, both a group effect and a decline with depth were evident (ANCOVA, $F_{1,99} = 6{\cdot}93$, $P < 0{\cdot}01$). The assumptions of normality and homogeneity of variances were met [Breusch–Pagan test: $BP_1 = 0{\cdot}05$ (benthics) $BP_1 = 0{\cdot}46$ (benthopelagics), $P > 0{\cdot}05$]. Despite homogeneity of variances (transformed data), it is important to note that the range of per cent red muscle appears very large at shallow mean depths and shrinks with increasing mean depth (Fig. 4). At shallow depths, species with the highest proportions of red muscle were found but so were species with little or no red muscle. Among the benthic group, the species in shallow water with high per cent red muscle were mostly flatfishes and a few gadids and among the benthopelagic fishes it was a carangid and a few gadiforms (Appendix).

The covariation between red muscle enzymatic activities and the proportions of red muscle was evaluated. None of the regressions between any of the four enzymes and the proportions of red muscle at either section 4 or 5 were significant ($P > 0{\cdot}05$).

DISCUSSION

Few studies have measured red muscle enzyme activities in demersal teleosts and those that have often used slightly different methodologies, which can affect comparisons. Johnston & Moon (1981) examined LDH and CS in six species and found very similar LDH activities. Their CS measurements were on the whole lower than those of this study, probably due to different assay methods (Condon *et al.*, 2012). Overall, the results of this study match those of others (Johnston & Moon, 1981; Moyes *et al.*, 1992; Yang & Somero, 1993) in that the activities of TCA enzymes were considerably higher than in white muscle for the same species and glycolytic enzyme activities were comparable or slightly higher to white muscle as well (Drazen & Seibel, 2007).

There were clear differences between the benthic and benthopelagic species' red muscle characteristics confirming a relationship to locomotory mode or overall level of activity (Greer-Walker & Pull, 1975; Johnston & Moon, 1981; McLaughlin & Kramer, 1991; Cediel *et al.*, 2008). Similar conclusions about proportions of red muscle were reached by McLaughlin & Kramer (1991) who used the terms 'sedentary' and 'mobile'. Their categories are similar but not identical to those used here. Their sedentary category included species that were principally described in natural history accounts as bottom dwelling. Their mobile category included true pelagic species that were not included in this study. Such broad dichotomies, however they are defined, are convenient particularly for deep-sea species where estimates of locomotory activity or capacity are rare, and broad inferences can still be helpful.

The results suggest that there is a reduction in routine locomotory ability with increasing depth but it is more subtle than Greer-Walker & Pull's (1975) assertion that deep-sea species are sluggish. CS activity declined significantly with mean depth of occurrence suggesting a decline in aerobic capacity of red muscle which should reduce routine swimming ability all else being equal. Nearly all of the data are

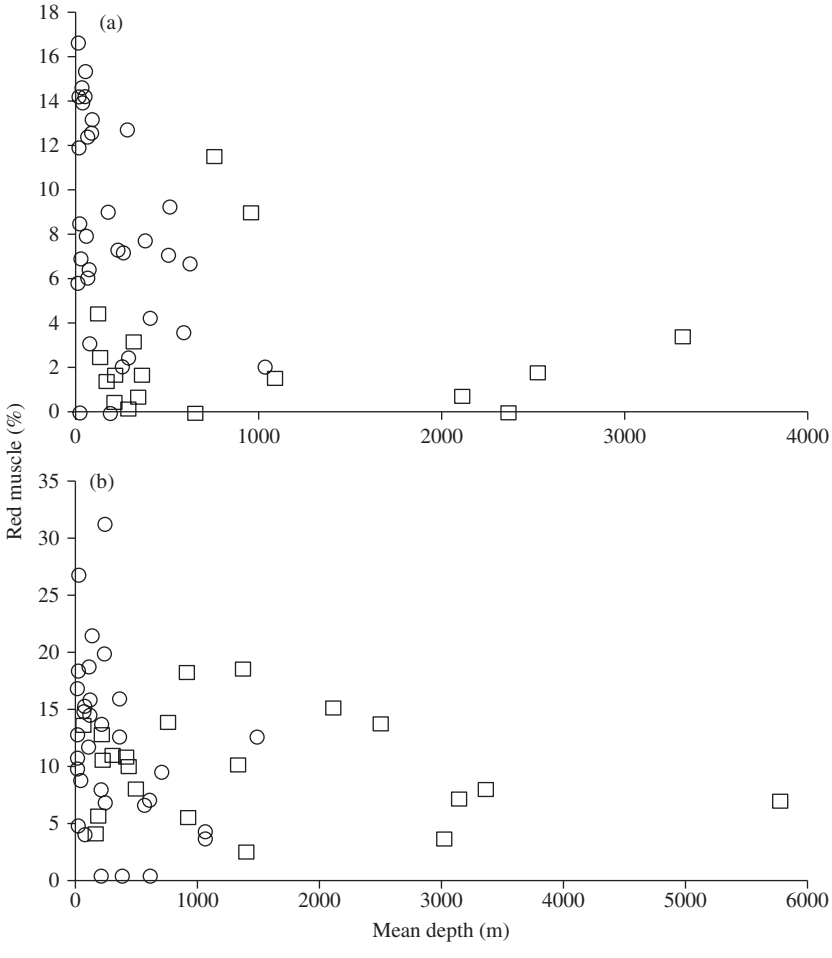

FIG. 4. Percentage of red muscle in (a) benthic and (b) benthopelagic fishes as a function of mean depth of occurrence. Values represent those generated from this study (□) and those from the literature (○).

for benthopelagic species. Yang & Somero (1993) found lower CS activities in deep-living *S. alascanus* compared to a shallow-living related species suggesting that at least some benthic species show the same pattern as benthopelagic ones. The proportions of red muscle also declined with depth in both benthic and benthopelagic species suggesting a reduction in routine locomotory activity. It is important to note that there is a great deal of variability in these values. For instance, in shallow water, there are benthic species with relatively large proportions of red muscle including flatfishes and benthic gadids (Greer-Walker & Pull, 1975; McLaughlin & Kramer, 1991; Appendix). Flatfishes can have relatively high metabolic rates (Duthie, 1982; Fonds *et al.*, 1992), which correlate to high activity levels (Dickson, 1995; Seibel & Drazen, 2007). It appears that less active species, as indicated by low proportions of red muscle, not only predominate at depth but are also present in shallow water. This pattern is suggested in the benthopelagic species as well. Despite a decline in mean proportions of red muscle with depth, sluggish species appear to exist at

all depths. The interesting question is why does the deep sea lack species with the highest indices of routine locomotory performance?

One framework for addressing this question is the visual interactions hypothesis (VIH) which has been proposed to explain depth-related declines in metabolic rate, white muscle protein content and enzyme activities in teleosts (Childress, 1995; Seibel & Drazen, 2007). Similar declines have been observed in white muscle of elasmobranchs (Treberg *et al.*, 2003; Condon *et al.*, 2012), cephalopods (Seibel *et al.*, 1997) and crustaceans (Childress *et al.*, 1990; Company & Sarda, 1998). The VIH reasons that as light levels decline sharply with depth so do the reactive distances between visually orienting predators and their prey. This results in a reduction in the selective pressure for high-speed locomotion, particularly in the pelagic where there are few opportunities for crypsis. Benthic fishes which could adopt cryptic strategies for predator avoidance or ambushing prey could exist at all depths so reductions in locomotory capacity would be less or non-existent. As a result of reductions in the locomotory apparatus, metabolism also declines. Burst locomotory performance involved in immediate predator–prey interactions is hypothesized to be reduced, which is corroborated by biochemical proxies of locomotion in white muscle. Declines in metabolic rates and metabolic enzyme activities in white muscle are most pronounced in pelagic, then benthopelagic and finally lowest in benthic species (Drazen & Seibel, 2007). Novel but limited *in situ* video studies of crustaceans (Bailey *et al.*, 2005*b*) and fishes (Bailey *et al.*, 2003, 2005*a*) attracted to bait suggest either lower or similar burst performance compared to shallow-living related species.

The VIH suggests that routine locomotory performance would generally remain the same regardless of light levels and depth. This is supported by red muscle enzyme data for elasmobranchs (Condon *et al.*, 2012) and by the finding that squid fin enzyme activities, which power slow undulatory swimming, do not change with depth (Seibel *et al.*, 1997). To some extent, routine and burst locomotory abilities are related to one another. One possible link between burst locomotory performance and red muscle physiology may occur *via* the oxidation of rapidly generated lactate. In teleost white muscle, most lactate is retained post-exercise where it is used in glyconeogenesis (Dickson, 1996; Gleeson, 1996). Ten to 20% of post-exercise lactate, however, is transported to the circulatory system and subsequently oxidized outside of the white muscle notably in the heart, liver and red muscle (Johnston & Moon, 1981; Gleeson, 1996; Sébert *et al.*, 2011). Given noted reductions in glycolytic capacity of white muscle with depth in these fishes (Drazen & Seibel, 2007), some reduction in red muscle CS activity might correspond to less need for rapid lactate oxidation. This potential role for red muscle would explain the depth-related decline in CS activity but it does not explain declines in proportions of red muscle.

Routine locomotion, powered by slow oxidative fibres, will include movements or station holding in the water column or at the bottom in the face of currents and turbulent flow. The physical energy of the marine environment, current velocities and turbulent flow, declines substantially with depth (Stabeno & Smith, 1987; Beaulieu & Baldwin, 1998). This could reduce the requirement for slow oxidative fibres used to maintain position or sustain a given rate of regular movement. This reduced requirement is likely to affect benthic species as well, as they must swim to relocate, forage and interact with other conspecifics. Hickey & Clements (2003) studied benthic (and one benthopelagic) triplefin fishes inhabiting a variety of nearshore habitats

with varying amounts of current. Slightly deeper-living species experiencing lower current speeds had less red muscle in cross section and had lower aerobic enzyme activities in their trunk musculature.

Another possible explanation for the observed patterns in red muscle morphology and enzymatic activity is a general change in body form of the fishes with depth. There is a preponderance of elongate body forms in the deep sea that has been noted for a long time (Marshall, 1954). Common deep-sea benthic and benthopelagic species include grenadiers, synaphobranchid eels, liparids, zoarcids and ophidiiforms all with anguilliform bodies (Merrett & Haedrich, 1997). Anguilliform locomotion is known to be four to six times more metabolically efficient at low speeds compared with other swimming modes (van Ginneken *et al.*, 2005; Tytell *et al.*, 2010). The drivers of this efficiency are as yet unclear. Neither propeller (biomechanical) nor muscle efficiency explanations are entirely satisfactory. Regardless of the causes, the apparent efficiency of anguilliform swimming and its prevalence at depth, broadly corresponds to the patterns noted in red muscle. It is possible that the proportions and perhaps enzyme activities of red muscle could decline and still maintain a level of routine locomotion, due to the economy of the propulsion form. Darkness and reduced demand for high-speed locomotion in predator–prey interactions would favour efficient slow speed swimming.

One study compared the more anguilliform swimming *C. armatus* and the subcarangiform swimming blue antimora *Antimora rostrata* (Günther 1878) at 2500 m depth in the North Atlantic Ocean (Collins *et al.*, 1999). Mean swimming speeds calculated from active tracking of *C. armatus* were *c*. 0·1 m s^{-1} (0·17 body lengths s^{-1}) compared with the faster *A. rostrata* at 0·21 m s^{-1} (0·39 body lengths s^{-1}). Tail beat frequency was also about twice as fast in *A. rostrata*. A Pacific congener with similar morphology (Small, 1981), finescale mora *Antimora microlepis* Bean 1890, has a metabolic rate about two-fold higher than that of *C. armatus* measured *in situ* off California (Drazen & Yeh, 2012) but its enzyme activities and proportions of red muscle are close to those of *C. armatus* (Tables I and II). It is impossible to compare efficiencies without measurements of metabolic expenditures. All that can be suggested is that *Antimora* spp. are much more active than *C. armatus*, and do so with similar amounts of red muscle and similar enzymatic capacities.

In conclusion, the novel results presented here on deep-sea fish red muscle proportions and enzyme activities suggest that there are declines in routine locomotory abilities with depth but sluggish species exist at all depths and there is an absence of the most active forms at greater depths. These results complement past work showing reduced metabolic rates in benthopelagic species (Drazen & Yeh, 2012) and reductions in white muscle protein and enzymatic activities with depth (Drazen & Seibel, 2007). Those patterns have been successfully explained with the VIH and while it might explain the patterns in red muscle characteristics, other factors such as reductions in physical energy with depth and differences in prevalent swimming modes may also be important.

We thank J. Sun for analysis of cross section images, R. Waller and I. Johnston for methodological advice with cross section analysis. A. Neuheimer assisted with tests for heteroscedasticity. E. Aus and N. Condon provided assistance with enzyme analysis. S. Ainsley, M. Boyle, D. Kline, C. Laxson, J. Lighten, K. Schmidt, P. Yancey and J. Yeh assisted with field collections. In addition to her support in the field, M. Kay provided educational outreach through her website http://deepblusea2009.blogspot.com/. Thanks to the captain and crew of

the R.V. *Point Sur*. This research was conducted in accordance with University of Hawaii Institutional Animal Care and Use Committee protocols. NSF provided funding for this work (OCE#0727135). This is SOEST contribution #8996.

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Appendix

Depths of occurrence and percentage of red muscle from literature sources used in depth regressions

Species	Family	% Red muscle	References	Lifestyle	Depth (m)			Depth references
					Minimum	Maximum	Mean	
Agonus cataphractus	Agonidae	0·0	(1)	B	0	370	185	(2)
Asterotheca pentacantha	Agonidae	0·2	(3)	B	110	454	282	(4, 5)
Alepocephalus tenebrosus	Alepocephalidae	10·3	(3)	BP	550	2100	1325	(4, 6, 7)
Anarhichas denticulatus	Anarchihidae	7·1	(1)	B	100	900	500	(2)
Anarhichas lupus	Anarchihidae	6·1	(1)	B	18	110	64	(2)
Anarhichas minor	Anarchihidae	7·2	(1)	B	100	400	250	(2)
Anoplopoma fimbria	Anoplomatidae	14·1	(3)	BP	200	1290	745	(8)
Epigonus telescopus	Apogonidae	16·3	(1)	BP	450	1100	775	(9)
Lepidorhombus whiffiagonis	Bothidae	4·3	(1)	B	100	700	400	(2)
Scophthalmus maximus	Bothidae	14·3	(1)	B	20	70	45	(2)
Callionymus lyra	Callionymiidae	0·0	(1)	B	5	30	18	(2)
Capros aper	Caproidae	0·6	(1)	BP	40	700	370	(2)
Caranx georgianus	Carangidae	27·0	(10)	BP	10	25	18	(2)
Conger conger	Congridae	3·6	(1)	B	0	1171	586	(2)
Icelinus tenuis	Cottidae	0·5	(3)	B	30	375	203	(4, 5)
Myoxocephalus scorpius	Cottidae	7·4	(1)	B	0	451	226	(2)
Taurulus bubalis	Cottidae	8·5	(1)	B	0	30	15	(2)
Cyclopterus lumpus	Cyclopteridae	18·9	(1)	BP	50	150	100	(2)
Brosme brosme	Gadidae	2·5	(1)	B	18	549	284	(2)
Ciliata mustela	Gadidae	16·7	(1)	B	0	22	11	(2)
Gadus morhua	Gadidae	21·7	(1)	BP	50	200	125	(11)
Gaidrposaurus vulgaris	Gadidae	12·5	(1)	B	10	120	65	(2)
Melanogrammus aeglefinus	Gadidae	16·0	(1)	BP	10	200	105	(2)
Merlangius merlangus	Gadidae	15·0	(1)	BP	30	100	65	(2)
Micromesistius poutassou	Gadidae	16·2	(1)	BP	300	400	350	(2)

Continued

Species	Family	% Red muscle	References	Lifestyle	Depth (m)			Depth references
					Minimum	Maximum	Mean	
Molva molva	Gadidae	2·1	(1)	B	100	400	250	(2)
Phycis blennoides	Gadidae	31·5	(1)	BP	150	300	225	(12)
Pollachius pollachius	Gadidae	4·3	(1)	BP	40	100	70	(2)
Pollachius virens	Gadidae	13·9	(1)	BP	37	364	200	(2)
Trisopterus luscus	Gadidae	15·5	(1)	BP	30	100	65	(2)
Trisopterus minutus	Gadidae	14·8	(1)	BP	15	200	108	(2)
Pomatoschistus microps	Gobiidae	5·8	(1)	B	0	12	6	(2)
Hemiramphus melanochir	Hemiramphidae	10·0	(10)	BP	0	20	10	(2)
Ophiodon elongatus	Hexagrammidae	1·7	(3)	B	10	408	209	(4, 5)
Girella tricuspidata	Kyphosidae	13·0	(10)	BP	0	20	10	(2)
Lophius piscatorius	Lophiidae	9·3	(1)	B	20	1000	510	(2)
Macrorhamphosus scolopax	Macrorhamphosidae	8·3	(1)	BP	50	350	200	(2)
Albatrossia pectoralis	Macrouridae	18·5	(3)	BP	500	1300	900	(13, 14)
Coelorinchus caelorhincus	Macrouridae	12·9	(1)	BP	200	500	350	(2)
Coryphaenoides acrolepis	Macrouridae	18·7	(3)	BP	700	2000	1350	(4, 5, 15)
Coryphaenoides armatus	Macrouridae	8·2	(3)	BP	2200	4500	3350	(15, 16)
Coryphaenoides filifer	Macrouridae	13·9	(3)	BP	2065	2904	2485	(17)
Coryphaenoides leptolepis	Macrouridae	3·8	(3)	BP	1998	4000	2999	(6, 15)
Coryphaenoides yaquinae	Macrouridae	7·2	(3)	BP	4500	7000	5750	(16, 18)
Nezumia aequalis	Macrouridae	0·6	(1)	BP	200	1000	600	(2)
Nezumia liolepis	Macrouridae	5·7	(3)	BP	581	1247	914	(19)
Trachyrhynchus trachyrincus	Macrouridae	3·9	(1)	BP	395	1700	1048	(2)
Merluccius merluccius	Merlucciidae	7·1	(1)	BP	70	400	235	(2)
Antimora microlepis	Moridae	15·4	(3)	BP	733	3000	2100	(4, 5, 11)
Lepidion eques	Moridae	9·7	(1)	BP	500	900	700	(2)
Mora moro	Moridae	12·9	(1)	BP	450	2500	1475	(2)
Aldrichetta fosteri	Mugilidae	17·0	(10)	BP	0	10	5	(2)
Chelon labrosus	Mugilidae	18·6	(1)	BP	0	25	12	(2)

Continued

Species	Family	% Red muscle	References	Lifestyle	Depth (m)			Depth references
					Minimum	Maximum	Mean	
Chilara taylori	Ophidiidae	2·5	(3)	B	10	246	128	(4, 5)
Spectrunculus grandis	Ophidiidae	7·4	(3)	BP	2000	4255	3128	(20–22)
Pholis gunnellus	Pholidae	14·3	(1)	B	0	30	15	(2)
Platycephalus bassensis	Platycephalidae	8·0	(10)	B	1	100	50	(2)
Atheresthes stomias	Pleuronectidae	3·2	(3)	B	20	600	310	(4, 5)
Citharichthys sordidus	Paralichthyidae	4·5	(3)	B	10	227	119	(4, 5)
Embassichthys bathybuis	Pleuronectidae	1·6	(3)	B	730	1430	1080	(5, 6, 23)
Errex zachirus	Pleuronectidae	0·7	(3)	B	20	650	335	(5, 23)
Glyptocephalus cynoglossus	Pleuronectidae	12·7	(1)	B	50	500	275	(12, 24)
Hippoglossoides platessoides	Pleuronectidae	9·0	(1)	B	90	250	170	(2)
Hippoglossus hippoglossus	Pleuronectidae	2·1	(1)	B	50	2000	1025	(2)
Limanda limanda	Pleuronectidae	13·2	(1)	B	20	150	85	(2)
Microstomus kitt	Pleuronectidae	12·6	(1)	B	10	150	80	(2)
Parophyrs vetulus	Pleuronectidae	1·4	(3)	B	20	310	165	(5)
Platichthyes flesus	Pleuronectidae	15·4	(1)	B	1	100	50	(2)
Pleuronectes platessa	Pleuronectidae	14·6	(1)	B	10	50	30	(2)
Genyonemus lineatus	Sciaenidae	13·8	(3)	BP	10	100	55	(5)
Gymnapistes marmoratus	Scorpaenidae	12·0	(10)	B	2	26	14	(2)
Helicolenus dactylopterus	Scorpaenidae	7·7	(1)	B	150	600	375	(2)
Sebastes aurora	Scorpaenidae	10·2	(3)	BP	80	770	425	(25)
Sebastes diploproa	Scorpaenidae	11·1	(3)	BP	200	600	400	(25)
Sebastes elongatus	Scorpaenidae	5·8	(3)	BP	100	250	175	(25)
Sebastes goodei	Scorpaenidae	13·0	(3)	BP	75	325	200	(25)
Sebastes jordani	Scorpaenidae	11·2	(3)	BP	90	490	290	(25)
Sebastes marinus	Scorpaenidae	6·9	(1)	BP	100	1000	550	(12)
Sebastes melanostomus	Scorpaenidae	8·2	(3)	BP	200	770	485	(25)
Sebastes mentella	Scorpaenidae	7·2	(1)	BP	300	900	600	(12)
Sebastes proriger	Scorpaenidae	10·8	(3)	BP	150	275	213	(25)

Continued

Species	Family	% Red muscle	References	Lifestyle	Depth (m)			Depth references
					Minimum	Maximum	Mean	
Sebastes saxicola	Scorpaenidae	4·2	(3)	BP	100	200	150	(25)
Sebastolobus alascanus	Scorpaenidae	11·5	(3)	B	300	1200	750	(25, 26)
Sebastolobus altivelis	Scorpaenidae	9·0	(3)	B	500	1400	950	(25, 26)
Trachyscorpia cristulata	Scorpaenidae	6·7	(1)	B	130	1100	615	(2)
Sillaginodes punctatus	Sillaganidae	5·0	(10)	BP	2	18	10	(2)
Sillago bassensis	Sillaganidae	9·0	(10)	BP	1	60	30	(2)
Buglossidium luteum	Soleidae	7·0	(1)	B	10	40	25	(2)
Solea solea	Soleidae	14·0	(1)	B	10	60	35	(2)
Acanthopagrus butcheri	Sparidae	11·0	(10)	BP	0	10	5	(2)
Pagellus bogaraveo	Sparidae	20·1	(1)	BP	150	300	225	(2)
Lumpenus lampretaeformis	Stichaeidae	6·5	(1)	B	40	100	70	(2)
Hoplostethus atlanticus	Trachichthyidae	4·5	(1)	BP	700	1400	1050	(9)
Trachinus vipera	Trachinidae	3·1	(1)	B	0	150	75	(2)
Lepidopus caudatus	Trichiuridae	0·6	(1)	BP	100	300	200	(2)
Zeus faber	Zeidae	12·0	(1)	BP	50	150	100	(2)
Bothrocara brunneum	Zoarcidae	2·7	(3)	BP	470	2300	1385	(4, 5)
Lycenchelys sp.	Zoarcidae	0·8	(3)	B	2100	2100	2100	capture depth
Lycodes cortezianus	Zoarcidae	1·7	(3)	B	70	644	357	(4, 5)
Lycodes diapterus	Zoarcidae	0·0	(3)	B	300	1000	650	(4, 5)
Pachycara bulbiceps	Zoarcidae	3·4	(3)	B	2600	4000	3300	(27)
Pachycara gymnium	Zoarcidae	1·9	(3)	B	1830	3200	2515	(27)
Pachycara lepinium	Zoarcidae	0·0	(3)	B	1730	2970	2350	(27)

B, benthic; BP, benthopelagic.

1, Greer-Walker & Pull (1975); 2, Froese & Pauly (2013); 3, Present study; 4, Lauth (2000); 5, Miller & Lea (1972); 6, Pearcy *et al.* (1982); 7, J. C. Drazen, unpubl. data; 8, Jacobson *et al.* (2001); 9, Francis *et al.* (2002); 10, Mosse & Hudson (1977); 11, Cohen *et al.* (1990); 12, Hureau (1996); 13, Clausen (2008); 14, Tuponogov *et al.* (2008); 15, Stein & Pearcy (1982); 16, Wilson & Waples (1983); 17, Endo *et al.* (1994); 18, Jamieson *et al.* (2009); 19, Hoff *et al.* (2000); 20, Mauchline & Gordon (1984); 21, Uiblein *et al.* (2008); 22, Monterey Bay Aquarium Research Institute; www.mbari.org/vars; 23, Vetter *et al.* (1994); 24, Bowering & Brodie (1991); 25, Love *et al.* (2002); 26, Jacobson & Vetter (1996); 27, Anderson & Peden (1988).

