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Red and white muscle proportions and enzyme activities in mesopelagic sharks

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ABSTRACT

In the last decade, there has been an increase in the study of the ecology of deep-sea organisms. One way to understand an organism's ecology is the study of its metabolism. According to literature, deep-sea sharks possess a lower anaerobic enzyme activity than their shallow-water counterparts, but no difference has been observed regarding their aerobic enzyme activities. These studies have suggested deep-sea sharks should be slow and listless swimmers. However, other studies based on video observations have revealed differences in cruise swimming speed between different species. The present study examined muscles of squaliform sharks, including both luminous and non-luminous species. We combined measurements of the relative amounts of red and white muscle with assays of enzymes that are used as markers for aerobic (citrate synthase, malate dehydrogenase) and anaerobic (lactate dehydrogenase) metabolism, searching for a relationship with cruising speeds. Non-luminous deep-sea species displayed lower aerobic enzyme activities but similar anaerobic enzyme activities than the benthic shallow-water counterpart (*Squalus acanthias*). Conversely, luminous Etmopteridae species were found to have similar aerobic enzyme activities to *S. acanthias* but displayed lower anaerobic enzyme activities. Analyses revealed that red muscle proportion and aerobic enzyme activities were positively related to the cruise swimming speed. In contrast, *Dalatias licha*, which swims at the slowest cruise swimming speed ever recorded, presented a very low aerobic metabolic phenotype (lower aerobic marker enzymes and less red muscle). Finally, the values obtained for white muscle proportion and anaerobic metabolic phenotype suggested a high burst capacity for *D. licha* and non-luminous sharks.

1. Introduction

The metabolism of an organism gathers all the reactions which take place in the cell of an organism, allowing it to synthesize organic material (anabolism) or to break molecules (catabolism) to provide energy. Most studies of organism's energetics focus more on catabolism. Studies of metabolic processes provide information on locomotion, growth, and feeding rates as well as activity patterns (Sullivan and Somero, 1980). Difficulties in accessing the deep-sea environment, hence getting animals in good physiological condition (most of them are dead or dying after capture), make the measurement of whole animal metabolism challenging if not impossible. Indirect metabolism measurements for deep-sea fishes using proxies such as enzyme activity assay represent a commonly applied method to bypass these difficulties (Drazen and Seibel, 2007). This method allows, under *in vitro* conditions, to measure as indices, the metabolism of animal tissues. However, these measurements are usually coupled with other metabolic proxies to get closer to the in vivo metabolism capacities. The aerobic and anaerobic metabolic pathways use substrate (fuel) to produce adenosine triphosphate (ATP) and metabolic building blocks that supply different needs. The aerobic pathway generates ATP via oxidation of nicotinamide adenine dinucleotide (NADH) while in the anaerobic one, the ability to sustain ATP production depends on lactate dehydrogenase oxidizing glycolytic NADH. The aerobic system produces more ATP per glucose molecule [38] than the anaerobic one [2] yet the former requires a higher concentration of oxygen and is one hundred times slower than the second pathway. Red muscle relies on aerobic metabolism to support activity. It is highly vascularized and contains a high level of mitochondria to provide steady and sustained effort (e.g., for cruise swimming). In contrast, while white muscle relies on aerobic metabolism in rest and recovery, anaerobic metabolism supports high intensity activity. It has few blood capillaries and contains a low level of mitochondria

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(Watanabe et al., 2012; Ryan et al., 2015). An intermediate type of fibre called pink fibre, is located between the red and the white muscles (Kryvi and Totland, 1977; Bone et al., 1986; Kiessling et al., 2006).

The measurement of enzyme activity is used as an index of aerobic and anaerobic capacity of a tissue. Thanks to the relative stability of enzyme activities in animals, this approach is essential in the study of the metabolism of elusive species (Drazen et al., 2015; Treberg et al., 2016). The metabolism of many deep-sea teleost fishes has been investigated in the last 40 years since enzyme activity correlates well with known or assumed metabolism in deep-sea and shallow water fishes (Torres et al., 1979; Childress and Somero, 1979; Sullivan and Somero, 1980; Drazen et al., 2011; Drazen et al., 2015). In contrast, only few studies have focused on the metabolism of elasmobranchs. Especially on the comparison between surface and deep-sea species. Most studies of deep-sea elasmobranch metabolism reveal a lower rate of anaerobic metabolism than their shallow-water counterparts (as with teleosts) but the aerobic phenotype is similar to shallow-water species (Treberg et al., 2003; Condon et al., 2012; Treberg et al., 2016). These studies have suggested that deep-sea sharks possess less efficient swimming capabilities than shallow-water species, based on two key hypotheses: (*i*) the lower water temperature inhibits muscular contraction (Treberg et al., 2003) and (*ii*) the reduced advantage of faster swimming capability in a low light environment; the visual interaction hypothesis (VIH) (Condon et al., 2012). However, these studies encompassed a limited number of deep-sea shark species along with skates, and the authors cautiously emphasized that generalities should not be made across this highly diverse group. A recent study using stereo-Baited Remote Underwater Videos (stereo BRUVs) has revealed differences in the cruise swimming speed of eight species of deep-sea Squaliformes sharks (Pinte et al., 2020). Results highlighted that luminous sharks from the Etmopteridae family displayed a higher cruise swimming speed than non-luminous counterparts. Indeed, etmopterids use the intensity of their ventral light to become invisible from prey and predator below; a strategy called "counter-illumination" camouflage (Claes et al., 2010; Claes and Mallefet, 2014). The organisms performing counter-illumination need to change light intensity quickly (Clarke, 1963; Young and Roper, 1976; Case et al., 1977; Latz, 1995). However, luminous sharks regulate their ventral light intensity by slow hormonal control (Claes et al., 2011; Claes and Mallefet, 2010, 2015; Duchatelet et al., 2019, 2020). Therefore, it has been suggested that luminous Etmopteridae may migrate up and down continuously in the water column to match their ventral light intensity with the downwelling light intensity of their environment, the "isolume-follower" hypothesis (Claes and Mallefet, 2014). In the present study, we investigated the metabolic phenotype of sharks through enzyme activity assays and red and white muscle proportion. A total of nine deep-sea species and one shallow-water one (*Squalus acanthias*) were analyzed. They are all members of the Squaliformes order, which allows reducing the potential phylogenetic difference observed. Aerobic metabolism is estimated using enzyme activity of citrate synthase (CS) and malate dehydrogenase (MDH). For anaerobic metabolism, we used the enzyme activity of lactate dehydrogenase (LDH). These enzymes were studied in red and white muscle samples and proportions of both muscular types according to the body mass were also measured. The aim was to explore the relationship between metabolic capacity and the cruise swimming speed (Pinte et al., 2020) in both luminous and non-luminous deep-sea sharks.

2. Materials and methods

2.1. Sampling

A total of ten shark species were collected from three different

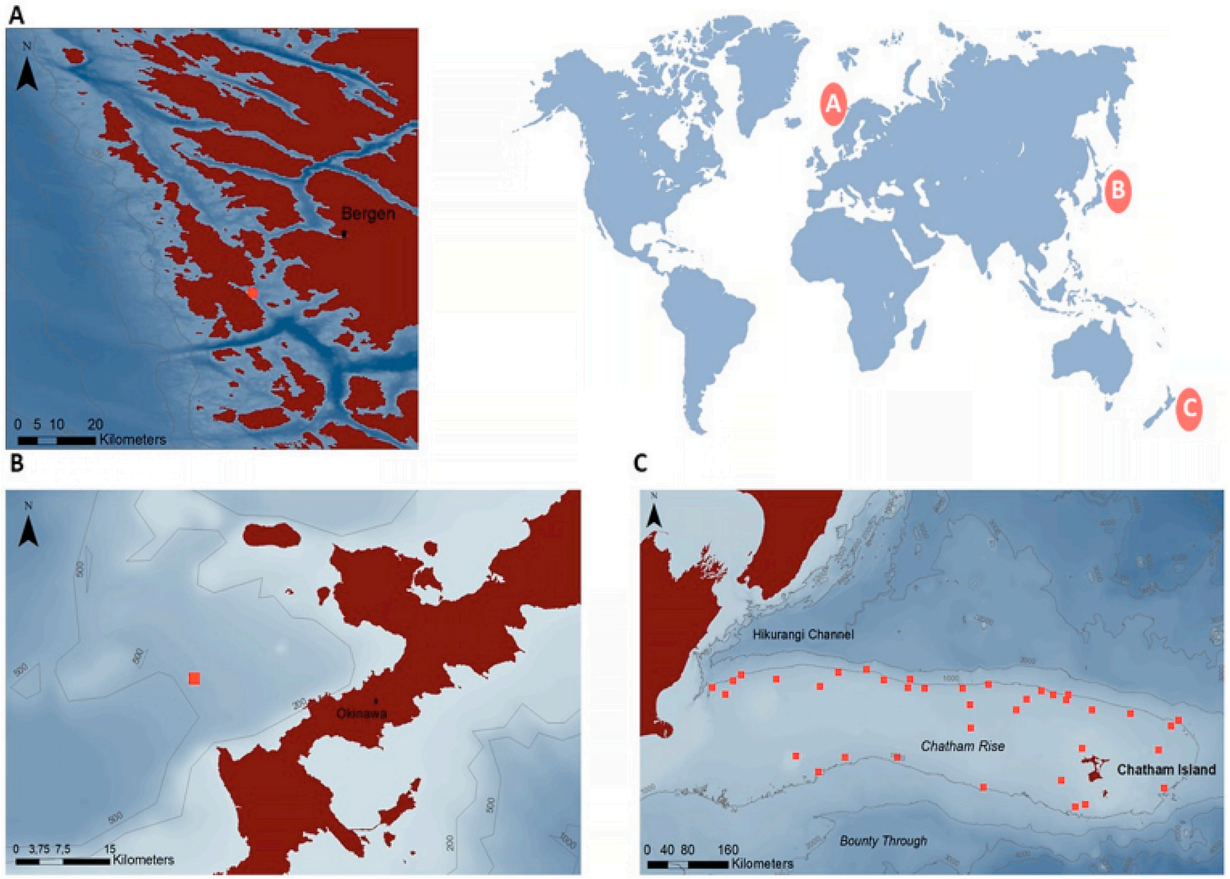

Fig. 1. Maps showing sampling location in Norway (A), Japan (B), and New Zealand (C).

locations. *Etmopterus spinax* was caught using long-line in Raunefjord (60.260623°N, 5.162056°E; Espegrend, Norway) (Fig. 1A), in January 2016 at depth of 225 m. Sharks were euthanized by a blow to the head followed by a full incision of the spinal cord at the level of the first vertebrae, following the local rules for experimental vertebrate care (Permit 12/14048). Animal procedures were conducted in compliance with the Belgian national guidelines and agreement with the European directive 2010/63/U, under the approval of the Animal Ethics Committee of the Université catholique de Louvain in Louvain-la-Neuve. Sharks were treated according to the European regulation for animal research handling and euthanized following the local rules for experimental vertebrate care.

Etmopterus molleri were caught on longlines during a survey in December 2016 at 500 m depth in the East China Sea (26.32468°N, 127.41207°E; Okinawa, Japan) (Fig. 1B). All shark specimens were euthanized according to the rules of OCA (Okinawa Churauchima Aquarium veterinary staff) for experimental fish care before experimentation took place.

A further eight species of shark, *Etmopterus granulosus*, *Etmopterus lucifer*, *Deania calcea*, *Centrophorus squamosus*, *Centroscymnus owstonii*, *Scymnodon plunketi*, *Dalatias licha* (deep-sea species), and *Squalus acanthias* (shallow-water species) were obtained as by-catch during a NIWA fisheries research survey for hoki on the Chatham rise, south-western Pacific in January 2018 (42.5907°S, 176.3978°E; New Zealand) (Fig. 1C).

All sharks selected (deep-water and shallow-water) are grouped in the Squaliformes order which allows a weak genetic diversity and slow rates of genetic change across familial and ordinal taxa (Treberg et al., 2003). Moreover, squaloids are morphologically homogeneous. They also display the same swimming mode, carangiform observed during video analyses (Pinte et al., 2020).

For each species, up to twelve specimens were sampled. White and red muscle samples were removed on ice directly after euthanasia. After removal, samples were immediately stored at −80 °C. The dissection work was done on ice with the shortest possible time between the manipulation and the storage at −80 °C to avoid enzyme degradation. Enzyme assays were performed on same samples over three-month period to evaluate if enzyme degradation occurred.

2.2. White and red muscle proportions

The protocol used to determine the red and white muscle proportions is based on Bernal et al. (2003a). Although previous studies have described intermediate muscular fibres in some elasmobranch species (Kryvi and Totland, 1977; Bone et al., 1986). In the present study, intermediate fibres were grouped with red ones since the visual quantification method does not allow the red and intermediate fibre distinction (Bernal et al., 2003a). For each species, three to four additional whole shark specimens were used for the red and white muscle quantification. All sharks studied exhibit a carangiform swimming mode (Ebert et al., 2013) which does not involve muscle at the anterior part of the body (Pinte et al., 2019). Therefore, the dissection protocol did not take into account the muscles found on the first 25% percent anterior and transverse section cuts started at a quarter of the fork length (FL), continuing to the caudal peduncle (85–95% FL). Each transverse section (5% of the fork length) was photographed on its anterior and posterior sides. Scaled photographs were analyzed with ImageJ2© software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997–2018). The red and white muscle volume for each cross-section is the product of the section thickness multiplied by its red/white muscle areas (mean between the anterior and the posterior area of the transverse section). The muscle mass was the product of muscle volume multiplied by density (literature value confirmed on our shark samples: 1.05 $g.cm^{-3}$, Bernal et al., 2003a). The total red and white muscle mass was determined by the sum of each section's mass values, these total muscle masses were standardized by the total body mass to calculate the red and white muscles proportion, % RM mass, and % WM mass respectively.

2.3. Enzyme assays

The maximal *in vitro* specific activity of the three enzymes in red and white muscles were used as proxies of the tissue's maximal *in vitro* metabolic capacity.The activities of the three enzymes, citrate synthase (CS - E.C. 4.1.3.7), malate dehydrogenase (MDH - E.C. 1.1.1.37), and lactate dehydrogenase (LDH - E.C. 1.1.1.27), were measured using a protocol based on those used in previous studies (Treberg et al., 2003; Condon et al., 2012). Results from *S. acanthias* were compared with published values to validate the methodology. No statistical differences were observed between the values we obtained and those reported in the cited studies (Student's *t*-test, $P > 0.05$). Frozen muscle samples were weighed (+/− 0.25 g) and homogenized using T-10 basic ultraTurax (IKA, Staufen, Germany) on ice in nine-volumes of cold 10 mM Tris-HCl buffer (pH 7.4 at 10 °C). The homogenates were centrifuged at 5000*g* for 5 min at 4 °C and the supernatants were used for the enzyme assays. To confirm that the homogenization of the tissue was effective, the protein concentration of each tissue supernatant was measured with the BCA assay (Bicinchoninic Acid assay).

All assays were performed at 10 °C in a total of 2.0 ml solution using a Genesis™ 10S UV–Vis spectrometer (ThermoFisher Scientific). The temperature was chosen based on water temperatures recorded at each station by CTD (Conductivity Temperature Depth). All shark species are known to experience this temperature during their lifetime. Before starting assays, it is essential to determine the saturating substrate concentration to obtain the maximal in vitro activity of the enzyme. For each species and tested enzyme, the substrate concentration was determined to be saturating when a linear decrease/increase of the substrate was observed after six minutes (i.e., two minutes after the enzyme activity measuring time in our protocol). After this first test the amount of tissue extract was doubled to verify that the absorbance variation was proportional to this increase of material (see Appendix 1). All measurements were carried out at one atmosphere. The enzyme rate is proportional to the decrease in absorbance at 340 nm (MDH and LDH) and the increase in absorbance at 412 nm (CS) with time. Results are expressed in international units (U): μmol of substrate converted to product per minute and per gram of wet tissue. The final conditions for each enzymatic assay were as follows: Citrate synthase: 100 mM Tris-HCl (pH 8.1), 0.1% Triton X100, 0.1 mM DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) and 0.15 mM of acetyl-CoA. The reaction was initiated by 0.5 mM oxaloacetate. Malate dehydrogenase: 100 mM Tris-HCl (pH 8.1); 20 mM $MgCl_2$ and 0.15 mM NADH. The reaction was initiated by 0.5 mM oxaloacetate. Lactate dehydrogenase: 80 mM Tris-HCl (pH 7.4); 8 mM pyruvate. The reaction was initiated by 0.10 mM NADH. A negative control, without cofactor and supernatant, confirmed that these two compounds are essential for the reaction start. Positive control with pure enzyme solution confirmed the enzyme's role in the measured reaction.

2.4. Statistical analyses

All statistical analyses were performed with JMP Pro 14 (JMP®, Version <14>. SAS Institute Inc., Cary, NC, 1989–2007). Normality and homoscedasticity of variance were confirmed for each parametric test (ANOVA, post hoc Tukey test). When the normality of variance was not apparent, equivalent non-parametric equivalent tests were used (Wilcoxon, post hoc Steel-Dwass test). Each difference was considered to be significant at a minimum $P < 0.05$. The resulting data on enzyme activity and muscle proportions were related to the cruise swimming speed using Principal Component Analysis (PCA), based on a pairwise Spearman correlation matrix. The swimming speeds used in this study were based on *in situ* observations of sharks using stereo BRUVs (Pinte et al., 2020).

3. Results

A total of 103 sharks were sampled. The size distribution, number of specimens by species, red/white muscle enzyme activities and proportion, and cruise swimming speeds from literature are summarized in Appendix 2.

3.1. Red and white muscle proportions

The analysis of the proportion of red muscle revealed significant differences between the studied shark species (Fig. 2A). The letters associated with each group indicate significant differences: when no letters are common, the groups are significantly different (ANOVA, $F(8,35) = 7.03$, $P < 0.05$, Tukey test post hoc). Group A is represented by *D. calcea* and *D. licha*; followed by Group ABC with *C. squamosus*, *E. lucifer*, *E. granulosus,* and *E. spinax*; then Group BC containing *C. owstonii* and *S. acanthias*; finally, Group C with only *E. molleri*.

The proportion of white muscle also varied between species with four statistically different groups identified (Fig. 2B), differences being lettered similarly as for the previous analysis (ANOVA, $F(8,35) = 7.37$, $P < 0.05$, Tukey test post hoc). The first group (A) is represented by *D. calcea*; the second group (AB) contained *C. squamosus, C. owstonii, D. licha*, and *S. acanthias*; the third group (BC) is represented by *E. granulosus* and *E. spinax*; finally, the last group (C) contains *E. lucifer* and *E. molleri.*

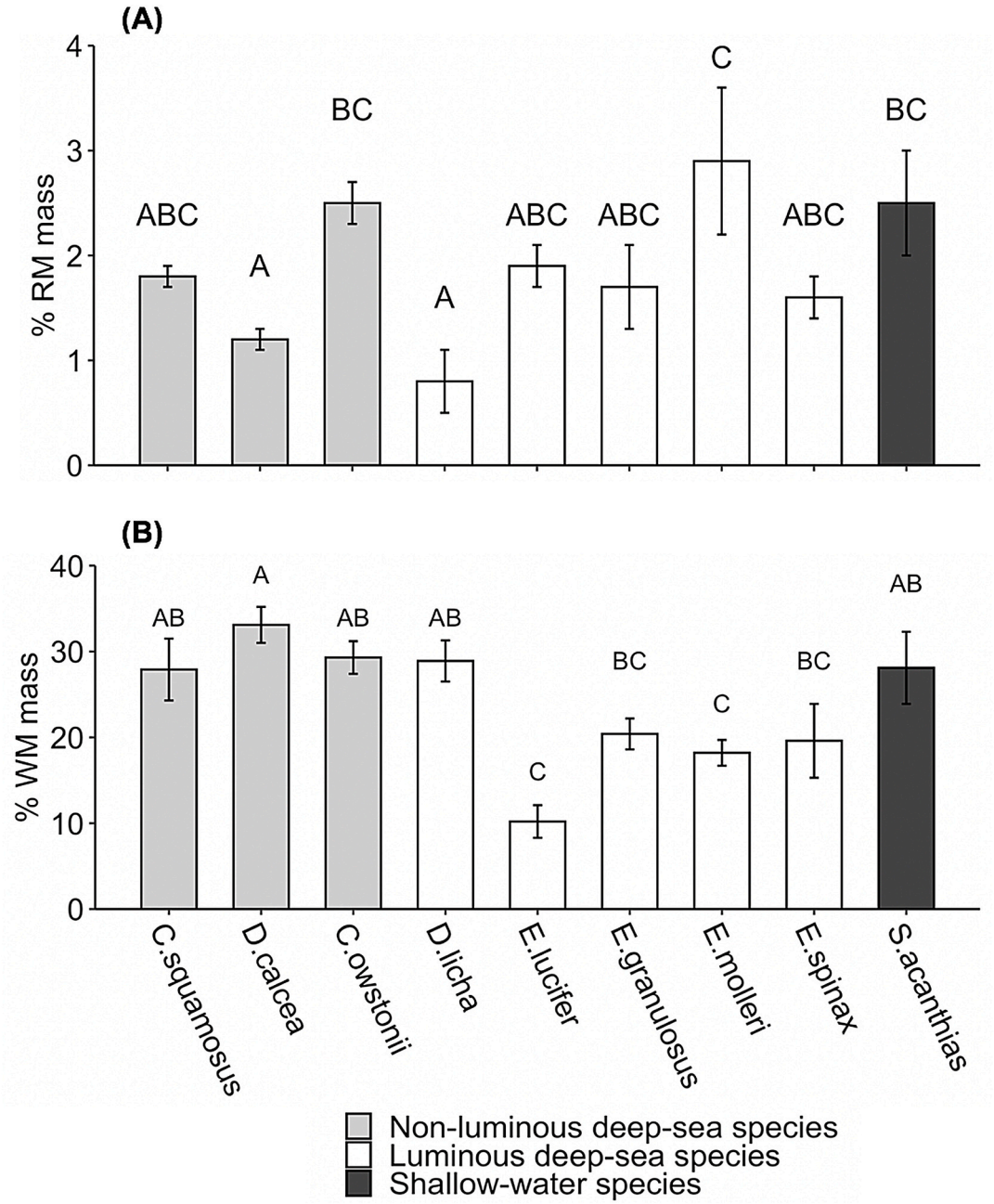

Fig. 2. Mean ± standard error of muscle proportions, corresponding to the muscle mass compared to the total body mass, of eight deep-sea sharks and one shallow-water species. (A) Red muscle proportion (RM% mass) (B) White muscle proportion (WM% mass). Letters above the bars describe the significant differences between species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Enzyme activities analyses

The activity of LDH in red muscle was lowest (36.1 ± 3.2 U) for the shallow-water species, *S. acanthias* (Fig. 3A) whilst deep-sea species displayed similarly high levels in their red muscle. The highest activity level (83.3 ± 5.4 U) was found in *E. granulosus* (ANOVA, $F(9,102) = 5.92$, $P < 0.05$, Tukey test post hoc). A gradient was observed in MDH activity in the red muscle (Fig. 3B), ranging from the lowest level observed in *D. licha* (39.3 ± 4.8 U) to the highest activity levels in *E. molleri* (178.2 ± 4.4 U). The other deep-sea shark species displayed intermediate MDH activity values, *C. squamosus* (B), *D. calcea* (BC), *S. plunketi* (BC), *C. owstonii* (BC), *E. lucifer* (BCD), *E. granulosus* (CD), *E. spinax* (DE), (ANOVA, $F(9,102) = 19.16$, $P < 0.05$, Tukey test post hoc), similar to the shallow-water species *S. acanthias* (91.8 ± 12.9 U). Finally, CS activity in the red muscle (Fig. 3C) showed two significantly different levels with a low activity group represented by *D. licha* (0.8 ± 0.2 U*), C. squamosus, D. calcea, S. plunketi, C. owstonii,* and the high activity group with *E. molleri* (12.6 ± 0.4 U), *E. lucifer, E. granulosus, E. spinax,* and *S. acanthias* (Wilcoxon, $H(9) = 80.56$, $P < 0.05$, Post hoc Steel-Dwass).

In white muscles, LDH activity (Fig. 4A) was grouped into two extreme values and one large intermediate group (ANOVA, $F(9,102) = 8.91$, $P < 0.05$, Tukey post hoc test). The lowest value was observed for *D. calcea* (0.4 ± 0.1 U), the highest value from *S. acanthias* (123.7 ± 16.0 U); the intermediate values recorde from the remaining species.

MDH activity levels were separated into four significantly different groups from the lowest to the highest level (Fig. 4B) (Wilcoxon, $H(9) = 62.63$, $P < 0.05$, Post hoc Steel-Dwass). The lowest activity level group (A) included *C. squamosus*, *D. licha* (19.6 ± 0.7 U), and *E. granulosus*. An intermediate group (AB) included *D. calcea, S. plunketi, C. owstonii, E. lucifer*, and *S. acanthias. A* third group (B) with slightly higher activity levels included only *E. molleri*; fand the highest activity was recorded in *E. spinax* (78.8 ± 3.7 U).

Statistical test distinguished three significant groups for CS activity levels (Fig. 4C) (Wilcoxon, $H(9) = 41.02$, $P < 0.05$, Post hoc Steel-Dwass). A low activity level group (A) included *S. plunketi* (0.1 ± 0.02 U) and *C. owstonii*; an intermediate group (AB) which included *C. squamosus, D. calcea, D. licha, E. granulosus*, and *E. molleri*; and a high activity level group (B) with *E. lucifer, E. spinax,* and *S. acanthias* (0.9 ± 0.2 U).

3.3. The relation between, muscle proportion, enzyme activities, and cruise swimming speed

A correlation between enzyme activity and cruise swimming speed was found for only six of the studied species. Principal Components Analysis results (Fig. 5 A-B) revealed a species dispersion mainly related to axis 1 (49.9%) and axis 2 (19.9%).

Both parts of the PCA (Fig. 5 A–B) showed a species dispersion mainly affected by muscle proportions, except for *C. owstonii,* isolated from other shark species due to its high LDH activity in white muscle. The loading graph (Fig. 5 B) showed a positive correlation between cruise swimming speed and the activity of CS and MDH in red muscle. According to the PCA results, white muscle proportion was negatively correlating with cruise swimming speed.

4. Discussion

4.1. Red and white muscle proportion (proportion of muscle to body mass)

The present study highlights a lower red muscle proportion for all but two ((*E. molleri* and *C. owstoni*) deep-sea shark species compared to their shallow-water counterpart (*S. acanthias*). Notably, these shark red muscle proportions are all lower than those of deep-sea teleost fishes (McLaughlin and Kramer, 1991; Bernal et al., 2003b; Drazen et al., 2013), the lowest proportion being recorded for *D. licha.* it is known that the liver comprises a much higher proportion of body mass in sharks, which may explain why sharks have a lower relative red muscle mass than teleosts. However, for teleost fish, the proportion of trunk red muscle is positively related to swimming activity (Drazen et al., 2013) whereas in sharks, it is related to buoyancy and swimming needs. Indeed, Bone's hypothesis proposes that sharks close to neutral buoyancy should have fewer red aerobic muscle fibres because their tailbeat only provides thrust, whereas negatively buoyant sharks use their tailbeat for thrust and lift (Bone, 1966; Shadwick and Goldbogen, 2012). In contrast to negatively buoyant shallow-water species, deep-sea sharks are thought to be neutrally or even positively buoyant as a results of their liver providing more lift (Bone and Roberts, 1969; Corner et al., 1969; Wetherbee and Nichols, 2000; Iosilevskii and Papastamatiou, 2016; Gleiss et al., 2017). Studying liver buoyancy in deep-sea sharks, Pinte et al. (2019) confirmed Bone's hypothesis by showing a negative relationship between red aerobic muscle proportions and the buoyancy provided by the liver. The observed low proprtion of red is in agreement with the buoyancy lift influence of the liver.

In contrast, the proportion of white muscle in deep-sea species was not different to the shallow-water shark species, except for the Etmopteridae, that displayed less white fibre than their deep-sea relatives. These results suggest that deep-sea sharks have identical muscular apparatus for burst swimming activity. However, the burst capabilities depend on many other factors such as metabolism, structural or physiological changes, etc.

4.2. Muscle enzyme activities

In previous publications, the red muscle aerobic activity has been described as relatively similar between chondrichthyan species from both deep-sea and shallow-water environments (Treberg et al., 2003; Condon et al., 2012; Treberg and Speers-Roesch, 2016). In the present study, the results suggested an alternative conclusion. Non-luminous species of deep-sea shark showed similar levels of MDH activity to *S. acanthias,* whereas the luminous Etmopteridae exhibited higher values. However, luminous *D. licha* displayed the lowest value of all species. Activity levels of the CS enzyme varied across all species without any clear separation between deep-sea and shallow-water groups or between luminous and non-luminous species. The LDH activities were quite similar between all deep-sea species but overall higher than the shallow-water species.

Regarding white muscle, the MDH activities were similar between all studied species except *E. molleri,* which displayed the highest value. The CS activities were different between all species but without any clear separation between deep-sea and shallow-water species, or between luminous and non-luminous species. Our results were in agreement with previous studies of both bony and cartilaginous fish, showing that the activities of these aerobic enzymes were significantly lower than in a red muscle (Childress and Somero, 1979; Sullivan and Somero, 1980; Treberg et al., 2003; Drazen et al., 2011; Condon et al., 2012). Red muscle is more vascularized, and can provide more oxygen for aerobic processes than white muscle (Kryvi and Totland, 1977). The number of mitochondria contained in these two types of muscular tissue plays a role; red fibres with more aerobically processes contain more mitochondria than white fibres (Kryvi and Totland, 1977). Finally, the differences in the myonuclear content and gene expression between white and red fibres could explain the difference of enzyme activities (Moyes et al., 1992).

In contrast, the LDH activity levels were low for *Deania calcea* and Etmopteridae species, whilst the other non-luminous species, *D. licha* and *S. acanthias* had higher values. LDH activity has been reported to decrease with the median depth occurrence (MDO) (Condon et al., 2012; Treberg et al., 2016). However, few deep-sea species were used in these previous studies. In the current study, results showed that some deep-sea sharks displayed values of enzymatic activity equivalent to anaerobic metabolism similar to *S. acanthias* (our shallow-water species reference).

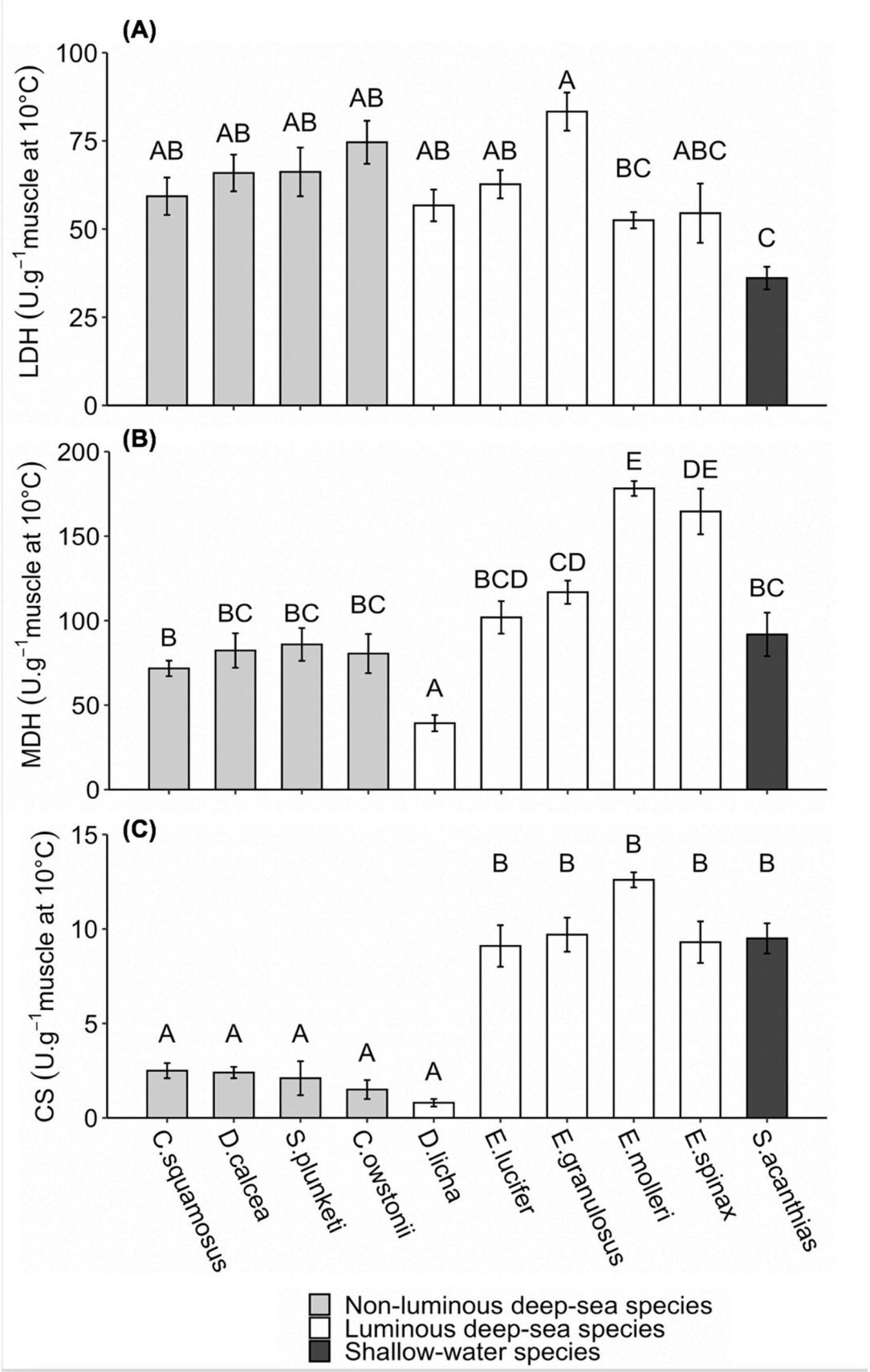

Fig. 3. Red muscle enzyme activities (mean ± standard error in international units per gram muscle at 10 °C) for (A) lactate dehydrogenase, (B) malate dehydrogenase, and (C) citrate synthase in nine species of deep-sea sharks and one shallow-water species (*S. acanthias*). Letters above the bars describe the significant differences between species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

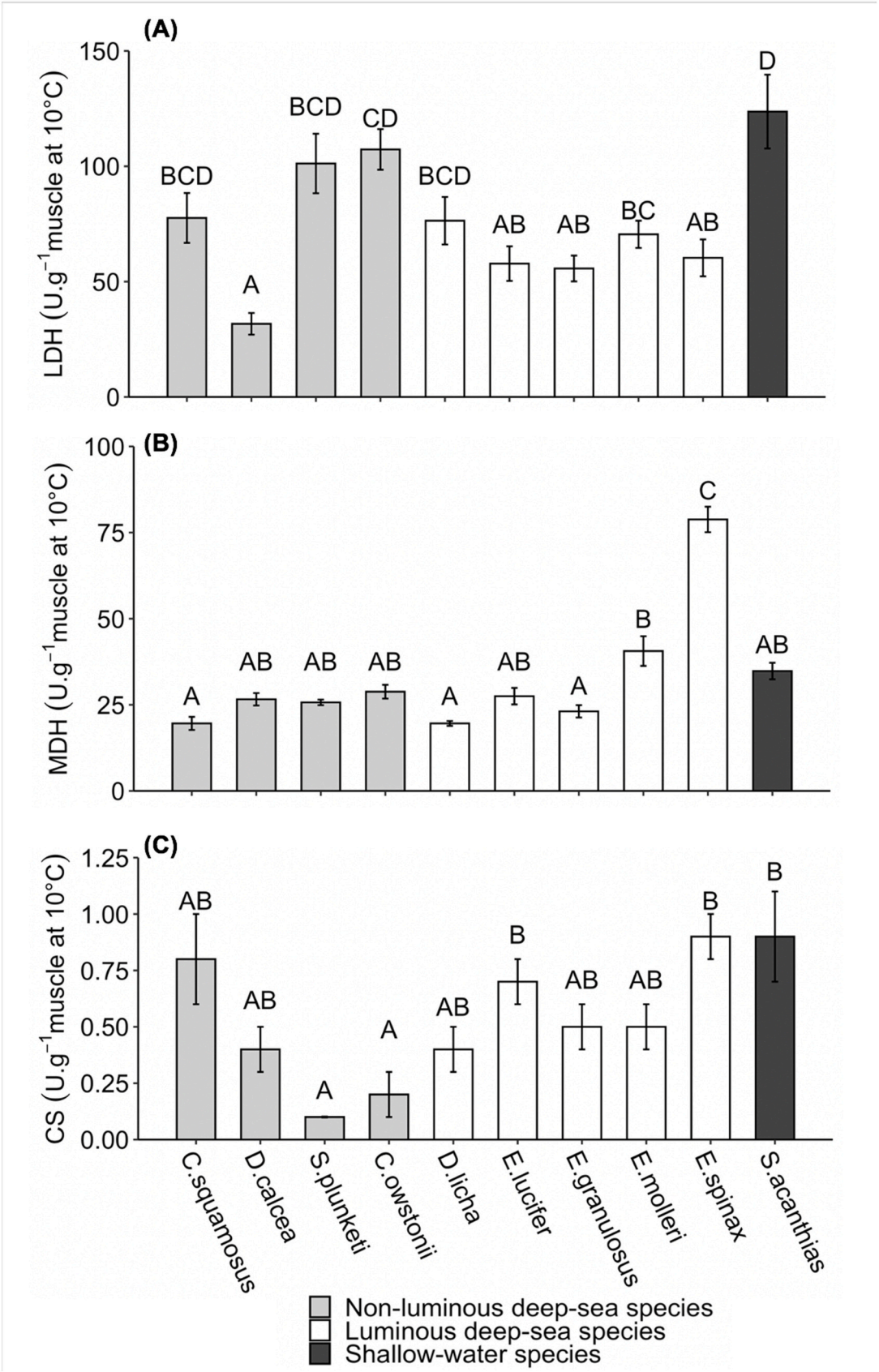

Fig. 4. White muscle enzyme activities (mean ± standard error in international units per gram muscle at 10 °C) for (A) lactate dehydrogenase, (B) malate dehydrogenase, and (C) citrate synthase in nine species of deep-sea sharks and one shallow-water species (*S. acanthias*). Letters above the bars describe the significant differences between species.

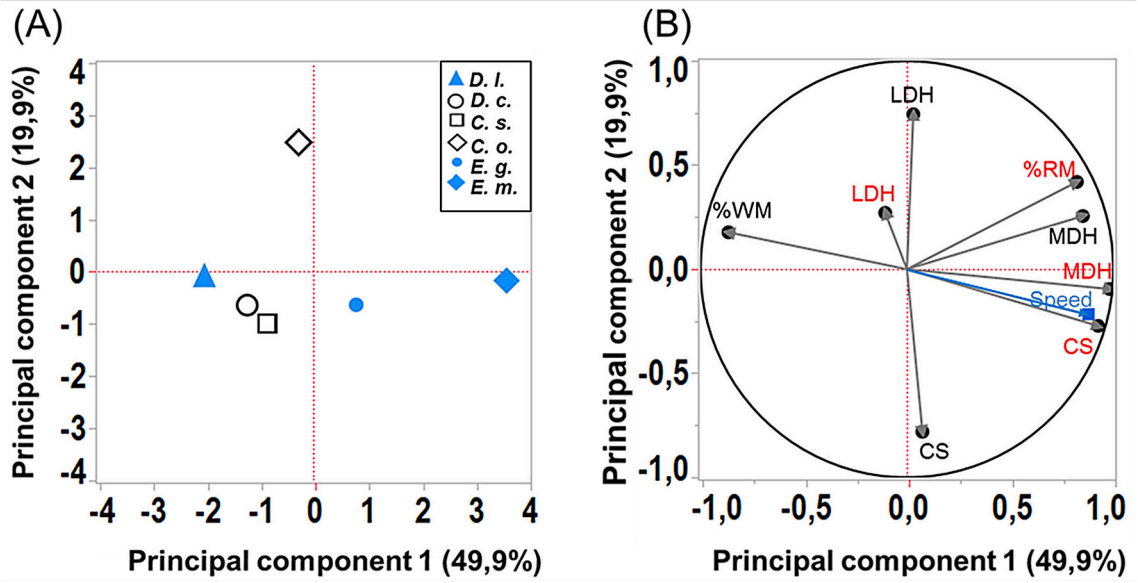

Fig. 5. (A) PCA score graph revealing species dispersion according to eight variables (speed is only a variable projection) where *D.l.* = *Dalatias licha, D.c.* = *Deania calcea, C.s.* = *Centrophorus squamosus, C.o.* = *Centroscymnus owstonii,* E.g. = *Etmopterus granulosus, E.m.* = *Etmopterus molleri.* Open symbols for non-luminous species, blue symbols for luminous species. (B) PCA loading graph illustrating the influence of eight variables on species dispersion; red colour corresponds to red muscle enzymes activities and proportion; black colour corresponds to white muscle enzyme activities and proportion; blue axis corresponding to the cruise swimming speed is a projection and does not take part in the species dispersion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

These values were low compared to those of pelagic fast-swimming sharks (Dickson et al., 1988; Dickson et al., 1993; Bernal et al., 2003a), however it is hard to compare enzymatic activities since the protocols for measurement of activity can vary. It is believed that enzymatic degradation during our protocol was unlikely. The enzymatic activities measured in the present study are similar or higher to the ones obtained by Treberg et al. (2003) and Condon et al. (2012), and enzyme assays performed on the same samples over the course of three months showed no significant differences. Moreover, enzyme assays performed on the same samples over three months showed no significant differences between assays made at different storage times. Comparison between the fast-swimming shallow-water sharks and the deep-sea squaliformes of our study might be tricky since they are phylogenetically distinct. The lowest value of LDH activity for *D. calcea* is difficult to interpret due to the paucity of information about this species. Only stomach content analyses are available to make any speculative hypothesis about the metabolism phenotype of white muscles of this species (Mauchline and Gordon, 1983; Daley et al., 2002; Dunn et al., 2013).

4.3. Relation with the cruise swimming speed

This study reports the first measurements of enzyme activities in red and white muscle of deep-sea species combined with the muscle proportions and known cruise swimming speeds. Principal component analysis revealed a strong positive relationship between the cruise swimming speed of deep-sea species and the CS - MDH activities in red muscle. Etmopterid species displayed the highest values of these two aerobic enzymes in red muscle, and also demonstrate the highest cruise speeds. It has been suggested that these species, which have a slow hormonal control of their bioluminescence, swim up and down in the water column to match their ventral light to that of their environment, the isolume follower's hypothesis (Claes et al., 2011; Claes and Mallefet, 2014; Claes and Mallefet, 2010, 2015; Duchatelet et al., 2019, 2020). The higher cruise swimming velocities measured in this family support this hypothesis (Pinte et al., 2020). Another explanation of high velocity may be the foraging behaviour and hunting risk. Etmopterid species perform vertical migrations to locate prey in the water column during the night while other deep-sea counterparts, such as *D. licha* forage mainly close to the deepsea floor (Mallefet et al., 2021). Finally, Etmopteridae are small species and may need to be active to avoid larger predators living in the same environment (Heithaus and Vaudo, 2012; Roberts et al., 2015; Pinte et al., 2020). The results in this study reveal possible physiological adaptations in aerobic phenotype to enable a higher cruise swimming speed. In contrast, *D. licha* swims at the slowest speed recorded for any shark, (Pinte et al., 2020; Mallefet et al., 2021) and the aerobic metabolism reported in this study, especially in the red muscle, was the weakest ever measured. This shark and other non-luminous deepwater sharks are thought to be ambush predators of smaller sharks such as the Etmopteridae (Navarro et al., 2014). These species should therefore display more effective burst swimming capabilities than Etmopterid species. The present results revealed a high proportion of white muscle with elevated LDH activity for *D. licha* and the other non-luminous species, which supports this hypothesis. *D. licha* has been observed to be capable of very fast (burst) swimming once close to its prey (see Zintzen et al., 2011 for footage and description of this behaviour). Further burst swimming speed observations will be required to reveal a potential correlation between anaerobic enzyme activities, white fibre proportion, and burst swimming capability.

5. Conclusions

Red and white locomotor enzyme activities of nine deep-sea shark species were measured as proxies of aerobic and anaerobic muscle metabolic capacity. Contrary to earlier studies, our results revealed that: (*i*) aerobic enzyme activities of Etmopteridae and the shallow-water species were similar, while non-luminous deep-sea species and *D. licha* had reduced values; (*ii*) anaerobic enzyme activity for Etmopteridae species was low whereas non-luminous deep-sea species and *D. licha* displayed similar values to *S. acanthias*. The difference in aerobic and anaerobic phenotype of the various species studied might reflect adaptations to specific behaviours. For Etmopterid species, the higher aerobic phenotype appears to be related to the reported cruise swimming speed, indicating a possible physiological adaptation to enable the "isolume-

follower" hypothesis. On the other hand, high white muscle proportion and anaerobic phenotype suggest strong burst capabilities for *D. licha* and non-luminous species.

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Ethical issues

Etmopterus spinax were collected in Norway under the "experimental fish care permit" number 12/14048. *Etmopterus molleri* were collected and handled according to Churaumi aquarium husbandry and veterinary rules for fish experimentations. All sharks were euthanized by a knock on the chondrocranium followed by an incision at the level of the spinal cord, following the local rules for experimental fish care and the European regulation for research animal handling. *Centrophorus squamosus, Deania calcea, Centroscymnus owstonii, Scymnodon plunketi, Dalatias licha, Etmopterus granulosus* and, *Squalus acanthias* were collected as by-catch species during the Hoki survey around the Chatham Rise in New Zealand. These species are not on the CITES list.

Data availability

The dataset of the current study is available from the corresponding author on reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpb.2021.110649.

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