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# Aerobic and anaerobic poise of white swimming muscles of the deep-diving scalloped hammerhead shark: comparison to sympatric coastal and deep-water species

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Scalloped hammerhead sharks (*Sphyrna lewini*) routinely perform rapid dives to forage on mesopelagic prey. These deep dives consist of intensive swimming followed by recovery periods in the surface mixed layer. Swimming muscle temperature profiles suggest that *S. lewini* suppresses gill function as a means to reduce convective heat loss during dives into cool water. Such intensive swimming behavior coupled with reduced respiration prompted us to test whether the aerobic and anaerobic metabolic capacities of the white swimming muscle tissue of this species are greater than those of other shark species from the same region. The activities of key enzymes used in aerobic and anaerobic metabolism provide an indirect indicator of the metabolic potential ("poise") of a tissue. Here we measured the maximal activities [international units (μmol substrate converted to product per min, U) per gram of wet tissue mass at 10°C] of the citric acid cycle enzymes citrate synthase (CS) and malate dehydrogenase (MDH) and glycolytic enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH) from white swimming muscle of *S. lewini*. Enzyme activities, and ratios of these enzyme activities that indicate relative indexes of aerobic to anaerobic capacity, were compared to those measured in three sympatric coastal carcharhinid sharks and two deep-dwelling species, *Echinorhinus cookei* and *Hexanchus griseus*. This is the first report of swimming-muscle enzyme activity for these deep-dwelling species. In comparison to the other species, *S. lewini* had significantly higher activities of both LDH and MDH in the white muscle, and a higher MDH/CS ratio. The high LDH activities suggest that the white muscle of *S. lewini* relies on relatively high rates of anaerobic ATP production, with would result in build up of high lactate levels, during deep foraging dives. High MDH activity in *S. lewini* white muscle suggests the potential for lactate levels to be rapidly reduced when aerobic

conditions are restored while in the surface mixed layer between dives. These biochemical characteristics may enable *S. lewini* to swim rapidly while suppressing gill function during deep dives and thereby exploit a very different ecological niche from sympatric shark species (e.g., coastal carcharhinids) and hunt more rapidly via faster swimming for deep-water prey compared to species that permanently inhabit deep depths.

KEYWORDS

**enzyme activity, lactate dehydrogenase, malate dehydrogenase, white muscle, *Sphyrna lewini*, *Hexanchus griseus*, deep-diving**

## Introduction

The scalloped hammerhead shark, *Sphyrna lewini*, shares overlapping daytime distributions in tropical and warm temperate coastal environments with other sympatric species (coastal carcharhinids), but exploits a very different ecological niche by foraging in deep, cold habitats. Scalloped hammerhead sharks conduct repetitive nocturnal deep dives (over 600m, sometimes exceeding 1000m) into cold water (as low as 5°C), presumably to forage on mesopelagic prey in the oxygen minimum zone (Jorgensen et al., 2009; Bessudo et al., 2011; Hoffmayer et al., 2013; Spaet et al., 2017; Anderson et al., 2022; Hutchinson et al., 2023; Royer et al., 2023). Royer et al. (2023) reported that *S. lewini* reduces convective heat loss at the gills during these deep dives, and suggested that this strategy of suppressing gill function is broadly similar to a "breath hold" dive. Biologger data of Royer et al. (2023) revealed that these deep dives are characterized by steep descents with swimming bursts when approaching maximum depth, intense activity throughout the deepest phase, with bottom times lasting an average of approximately 4 minutes, and a consistent high-frequency, high-amplitude tailbeat during ascent (Figure 1). During the ascent, at a depth of ~250m, swimming intensity decreases abruptly, sharks level out and ascend more slowly. This part of the ascent is characterized as the "inflection point." On reaching the surface mixed layer, sharks swim using a lower tailbeat frequency and amplitude until starting the next deep dive. Overall, these dives last an average of 56 minutes (from the start of the slow descent to the end of the slow ascent), with the high-activity phase of the dives (from the start of the fast descent to the end of the fast ascent at the inflection point) lasting an average of 17 minutes. The sharks stayed within the top 50 meters of the water column during their interdive intervals which lasted an average of 43 minutes and ranged from 18 minutes to >3 hours. Temperature measurements of the white swimming muscle show that *S. lewini* stays warm throughout the deepest portion of the dives and substantive cooling only occurs during the latter stages of the ascent phase and, once initiated, is rapid. These rapid changes in body temperature at different points of the dive cannot be explained by simple thermal inertia. Modeling of heat transfer coefficients (Royer et al., 2023) indicates that convective heat transfer at the gills is suppressed during the high-activity phase of the dives. Although the active mechanism for reducing heat loss at the gills is unknown (e.g., shunting blood away from the gills, reducing blood flow to the gills, reducing ram ventilation by closing the mouth, gill slits or both), any of these will inhibit the shark's ability to absorb oxygen from the environment. Video of a scalloped hammerhead shark swimming along the seabed at a depth of 1,044m showed its gill slits tightly closed, whereas similar images from surface waters show these sharks swimming with their gill slits wide open, supporting the gill-slit-closing hypothesis (Moore and Gates, 2015; Royer et al., 2023). A sudden cooling in muscle temperature as scalloped hammerhead sharks approach the surface at the end of each dive suggests that they have opened their gill slits to resume gill ventilation while still in relatively cool water.

Powering swimming during these repetitive, intense, deep dives necessitates a high energetic output by the locomotor muscles. It is possible that *S. lewini* relies on anaerobic energy production during these deep dives due to the combination of intense swimming activity and suppression of normal respiration. This highly active swimming combined with apparent "breath holding" should be reflected in the activities of key muscle enzymes involved with locomotion and energy mobilization.

Anaerobic metabolism becomes an essential process when aerobic pathways of ATP production cannot sustain cellular energetic demands (Pörtner, 2002). Burst swimming typically requires anaerobic metabolism and a greater reliance on muscle energy stores (Williams et al., 1997). In fishes, fast-twitch glycolytic white (type II) muscle is the largest tissue mass and is specialized for anaerobic high-intensity swimming when power output is needed beyond what the slow-twitch red muscle (type I) is capable of producing (Bernal et al., 2003; Seamone and Syme, 2016). High-intensity swimming events, such as the chasing of prey, typically require anaerobic pathways independent of oxygen availability and deplete muscle creatine-phosphate and glycogen stores, leading to a concomitant buildup of lactate and $H^+$ byproducts (Guppy and Hochachka, 1978; Bernal et al., 2003; Kane, 2014).

Generally, elasmobranchs found in warm shallow waters (above mesophotic depths) have higher metabolic rates and stronger burst-

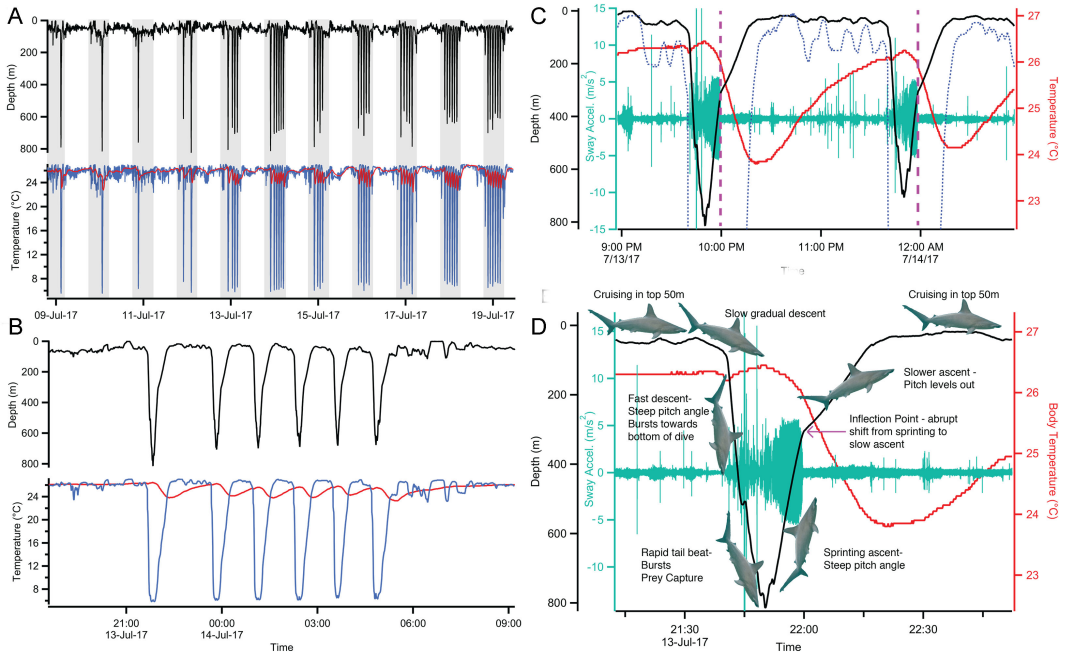


FIGURE 1
Deep-diving behavior and body temperature of *S. lewini*, reproduced from Royer et al. (2023). Ambient (blue), and intramuscular (red) temperature profiles from a scalloped hammerhead shark deep-diving during nighttime **(A)**, with repetitive deep-dives shown during a single evening **(B)**. **(C)** Depth profiles (black), body temperature (red), ambient water temperature (blue dashed), and swimming activity [tailbeat sway acceleration (teal)] during two successive deep dives. Dotted lines indicate the inflection point of the ascents when the swimming activity and pitch angle abruptly decrease. **(D)** Distinct phases of a deep dive.

locomotor capabilities relative to deep-dwelling elasmobranchs (Treberg et al., 2003; Condon et al., 2012). This is supported by electronic tagging data, with slower vertical velocities, slower swimming speeds, and slower tail beats for *H. griseus* and *E. cookei* than for epipelagic shark species (Nakamura et al., 2011, 2015; Comfort and Weng, 2015; Andrzejaczek et al., 2018; Coffey et al., 2020; Royer et al., 2023). Capacity of tissues for aerobic and anaerobic metabolism can be estimated by measuring the maximal activity of key enzymes involved in the production of ATP. The activities of the enzymes citrate synthase (CS) and malate dehydrogenase (MDH) have been widely used as indicators of tissue aerobic capacity. CS is the first catalyst in the citric acid cycle (Childress and Somero, 1979) and its activity correlates with tissue mitochondrial density (Moyes et al., 1992; Dickson et al., 1993; Dickson, 1995; Duong et al., 2006) and whole animal metabolic rate (Torres and Somero, 1988; Hochachka and Somero, 2002). MDH is used in the citric acid cycle in the shuttling of reducing equivalents between the mitochondria and cytosol. MDH is also involved in gluconeogenesis, the synthesis of glucose from smaller molecules such as pyruvate formed by oxidation of lactate accumulated during anaerobic function (Kane, 2014; Rogatzki et al., 2015). Activities of the enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH) have been used as indicators of anaerobic capacity. PK is a good indicator of the capacity for glycolysis, as it catalyzes pyruvate formation during glycolysis (Somero and Childress, 1980). LDH catalyzes the reversible conversion of pyruvate and NADH to lactate and $NAD^+$ to maintain redox balance, allowing anaerobic production of ATP to continue in the cytosol; therefore, LDH activity is considered a strong indicator of tissue anaerobic capacity (Hochachka et al., 1982). Measurements of the maximal activities of all four of these enzymes have been widely used in previous studies to assess the aerobic and anaerobic capacity of muscle in fishes (Childress and Somero, 1979; Sullivan and Somero, 1980; Somero, 1992; Dickson et al., 1993; Vetter and Lynn, 1997; Bernal et al., 2003; Treberg et al., 2003; Ombres et al., 2011; Condon et al., 2012; Drazen et al., 2015; Saavedra et al., 2016; Pinte et al., 2021).

The combination of intensive swimming and likely suppressed gill function suggests that *S. lewini* relies heavily on anaerobic metabolism during deep dives (Meekan and Gleiss, 2023). These characteristics may enable *S. lewini* to swim rapidly while suppressing gill function during dives and thereby exploit a very different ecological niche from sympatric shark species (e.g., coastal carcharhinids) and hunt more rapidly for deep-water prey compared to slow-moving shark species that permanently inhabit deep depths. Therefore, we predicted that the white muscle tissue of *S. lewini* possesses enzyme characteristics that facilitate anaerobic metabolism during deep dives, and the necessary aerobic metabolism to allow for rapid recovery (i.e., the breakdown of anaerobic end products) in well-oxygenated surface waters during intervals between dives. Furthermore, we predicted that the white muscle enzyme characteristics of *S. lewini* can be distinguished from those of both sympatric shallow-water shark species (e.g., coastal carcharhinids) that share similar daytime distributions and deep-water shark species, by having a greater capacity for both anaerobic and aerobic metabolism in the white swimming muscle. To test this

hypothesis, the maximal activities of the enzymes CS, MDH, PK, and LDH were measured to assess the aerobic and anaerobic poise of the white muscle of adult *S. lewini*. Activities of these enzymes were compared to those measured in other coastal tropical/temperate shark species [sandbar shark (*Carcharhinus plumbeus*), blacktip shark (*Carcharhinus limbatus*), and tiger shark (*Galeocerdo cuvier*)] and in two deep-water sharks [bluntnose sixgill (*Hexanchus griseus*) and prickly shark (*Echinorhinus cookei*)].

# Materials and methods

## Tissue sampling

White muscle tissue samples were collected from sharks caught on demersal long-lines set in waters off the island of O'ahu (Hawai'i, USA). Shallow species (*S. lewini, G. cuvier, C. plumbeus, C. limbatus*) were caught at depths of 5-50 meters and deep species (*H. griseus* and *E. cookei*) were caught between depths of 250–300 meters. Muscle samples were collected from a 2-cm incision below the base of the first dorsal fin using a 5mm or 8mm biopsy punch. Samples were placed in a cryovial and immediately dropped into a Dewar flask containing liquid nitrogen. Samples were later transferred to and stored in a -80°C freezer for 3-24 months. This storage time falls within the allowable timeframe between tissue sampling and assay running without compromising (decreasing) tissue enzyme activity (Dickson et al., 1993; Condon et al., 2012).

## Enzyme assays

The maximal activities of the enzymes CS, PK, LDH, and MDH in each white muscle tissue sample were measured using enzyme assay protocols based on those established by previous studies (Childress and Somero, 1979; Treberg et al., 2003; Condon et al., 2012; Friedman et al., 2012). Frozen muscle samples were weighed and homogenized in a Kontes Duall ground glass tissue grinder with ice-cold 10 mM Tris-HCL buffer (pH 7.55 at 10°C) at a ratio of 0.1 g of tissue to 1 ml of buffer. Duplicate homogenates were prepared for each sample unless the total sample was less than 0.05 g, in which case a single homogenate was prepared. CS assays were performed before centrifugation for the other enzyme assays. Homogenates were then centrifuged at 5000*g* for 5 minutes, and the supernatants were used in the assays.

All assays were run in a volume of 2 mL at 10°C in a Shimadzu UV 1601 spectrophotometer with a water-jacketed 12-cell cuvette holder attached to a water chiller (Condon et al., 2012). Activities were measured as change in absorbance over time and reported in international units (U; µmol substrate converted to product per min) per gram of wet tissue mass. PK, LDH, and MDH assays were run at 340 nm for 40 seconds and CS was run at 412 nm for 3 minutes. Enzyme assays were run under the following saturating substrate conditions: citrate synthase: 0.1 mM 5,5-dithiobis-nitrobenzoic acid (DTNB), 50 mM imidazole HCl (pH 8.0 at 10° C), 2 mM $MgCl_2$, 0.1 mM acetyl-CoA, 0.5 mM oxaloacetate. After recording background change in absorbance over time with the tissue homogenate present, the CS reaction was initiated by adding oxaloacetate substrate. Pyruvate kinase: 80 mM Tris HCl (pH 7.8 at 10°C), 100 mM KCl, 10 mM $MgSO_4$, 10 U/ml lactate dehydrogenase, 0.1 mM fructose 1-6 bisphosphate, 5.0 mM adenosine diphosphate, 150 µM NADH. The PK reaction was initiated by the addition of 1.0 mM phosphoenol pyruvate. Lactate dehydrogenase: 80 mM imidazole (pH 7.8 at 10°C), 100 mM KCl, 150 µM NADH, 2 mM sodium pyruvate. Malate dehydrogenase: 100 mM Tris HCl (pH 8.1 at 10°C), 20 mM $MgCl_2$, 150 µM NADH, 0.5 mM oxaloacetic acid. LDH and MDH reactions were initiated with the addition of the supernatant.

## Data analysis

The body mass for each shark was estimated from measured lengths using length-weight relationships from Kohler et al. (1996) for *S. lewini, G. cuvier, C. plumbeus*, Ebert (1986) for *H. griseus*, and Pollack et al. (2019) for *C. limbatus*. No length-weight relationships were available for *E. cookei*. Regression analysis was used to evaluate whether muscle enzyme activity scaled with shark body size for each species. Normality of enzyme activity data was assessed by examining distribution histograms for each species and enzyme, and using Levene's test to assess homogeneity of variance. Due to the non-normal (based on histograms and NQQ plots) and heteroscedastic (Levene's test, all $P < 0.05$) nature of the data and the unequal sample sizes among species, non-parametric Welch's ANOVAs with *post-hoc* Games-Howell tests were used to assess interspecific differences in muscle enzyme activity. High activity of MDH observed in *S. lewini* prompted a *post-hoc* interspecific comparison of the ratio of the aerobic enzymes (MDH and CS). The ratio of MDH to CS activity was calculated for each individual shark, and then averages and standard deviations were calculated for each species. Based on similar data characteristics mentioned above, non-parametric Welch's ANOVA with a *post-hoc* Games-Howell test was used to assess interspecific differences in the MDH: CS ratio between species.

# Results

## Body size scaling effects on enzyme activity

All *S. lewini* (n = 10) and *E. cookei* (n = 5) sampled were mature adult males. All *G. cuvier* (n = 22) were immature sub-adults or juveniles. One *C. limbatus* (n = 5) and 12 C. *plumbeus* (n = 24) were mature adults. Body length measurements were missing for 2 *G. cuvier* and 3 C. *plumbeus*. Two of the male *H. griseus* (n = 9) were mature adults, 2 of the other males were large sub-adults, and all 5 of the females were large sub-adults, based on clasper calcification (for males) and estimates of age at maturity from (Ebert, 2002). Regression analyses showed no significant scaling of enzyme activity with body size for the species studied (all $P$ values > 0.05), with the exception of LDH and MDH for *S. lewini* ($P$ = 0.02, $P$ = 0.01) and *H. griseus* ($P$ = 0.03, $P$ = 0.02). The largest

individual of both species had unusually low LDH and MDH values. When those two individuals were removed as outliers, no significant body size scaling was observed (all P values > 0.05). As a result, mean enzyme activities, unadjusted for body size but with the outlier data included, are reported in Table 1 and used for interspecific comparisons.

## White muscle enzyme activities

*Sphyrna lewini* white muscle exhibited high activities of LDH, CS, and MDH (Table 1), with the activities of LDH and MDH of *S. lewini* standing out as much higher in comparison to the sympatric coastal (*C. plumbeus*, *C. limbatus*, *G. cuvier*) and deep-water (*H. griseus*, *E. cookei*) species (Figures 2, 3A). MDH activity in *S. lewini* was significantly greater than that in all of the coastal and deep-water species (all $P \leq 0.007$, Games-Howell tests; Figure 1). LDH activity was significantly greater in *S. lewini* than in the coastal sharks *C. limbatus* ($P = 0.02$) and *C. plumbeus* ($P = 0.003$) and the deep-water shark *H. griseus* ($P = 0.006$). Average CS activity in *S. lewini* was the highest amongst the species studied, but not significantly so (all $P > 0.05$) (Figure 2). PK activity in *S. lewini* did not differ significantly from that measured in the coastal sharks *C. limbatus* and *C. plumbeus* and in both deep water sharks, but was significantly lower than that of *G. cuvier* ($P < 0.001$). MDH activity relative to CS activity was highest in *S. lewini* compared to the other coastal carcharhinids and bathyal shark species, followed by *C. plumbeus* and *G. cuvier* (Table 2, Figure 3B). Though the statistical comparisons of MDH: CS ratios between *S. lewini* and all other species except *E. cookei* were not significant (all $P>0.05$), it is noticeable in Figure 3B that *G. cuvier* and *C. plumbeus* also have high CS activity but not the correspondingly higher MDH activity observed in *S. lewini*.

# Discussion

Based on its higher LDH and MDH activities (Figure 3A), and to a lesser extent the relatively high CS activity (Table 2, Figure 3B), *S. lewini* white muscle has higher anaerobic and aerobic capacities than other coastal carcharhinids and bathyal shark species that do not exhibit the repetitive nocturnal dives observed in *S. lewini*. Analyses of white muscle metabolic poise suggest that *S. lewini* possesses enzyme characteristics that facilitate high anaerobic metabolic activity during deep dives and the necessary aerobic metabolism to allow for rapid recovery, through the breakdown of anaerobic end products, when near the surface between dives. These physiological characteristics are proposed to enable the observed high activity levels of *S. lewini* even while they are suppressing gill function during dives into cold habitats. These same features would allow them to recover quickly in well-oxygenated surface waters through rapid processing of the lactate built up during anaerobic activity (Meekan and Gleiss, 2023). The intense swimming activity exhibited by *S. lewini* during deep dives requires ATP production at a high rate (Meekan and Gleiss, 2023). It is unknown to what extent anaerobic ATP production in the white muscle is required during dives, but the high intensity swimming, including frequent bursts, recorded by Royer et al. (2023) suggests that the white fast-twitch muscle is active at depth, making it likely that anaerobic metabolism and lactate production occur. This would then require the lactate to be removed from the white muscle or possibly processed within it after a dive. It is possible that red slow-twitch muscle is also recruited during the rapid but sustained swimming phases of each deep dive, reducing to some extent the amount of lactate produced in the white muscle. Regardless of the extent to which deep-diving behavior relies on aerobic versus anaerobic capacity, these behavioral and physiological characteristics likely enable *S. lewini* to exploit a very different ecological niche than other sympatric shark species (e.g., coastal carcharhinids with daytime distributions overlapping that of *S. lewini*).

The white muscle enzyme profile of *S. lewini* suggests that the deep-water hunting capacity of this species may be facilitated by rapid recovery in surface waters between dives. Higher levels of LDH allow for higher rates of glycolysis to power white muscle contraction during bursts and high-speed swimming, but can also cause a high rate of lactate buildup in the cytosol. The amount and fate of lactate produced in the white muscle of *S. lewini* during intensive swimming during dives is unknown. Previous studies indicate that teleosts and the dogfish shark, *Squalus acanthias*, retain lactate produced from strenuous exercise in their white muscle (for up to 4 hours in *S. acanthias*) (Milligan and Wood, 1986; Girard and Milligan, 1992; Richards et al., 2003), but little evidence for gluconeogenesis within fish white muscle has been found. Lactate can be converted to pyruvate and then used as a substrate for ATP synthesis or in gluconeogenesis under aerobic

TABLE 1 White muscle enzyme activity (mean ± standard deviation) reported in U/g wet mass at 10°C for each species.

| Species | N | BW Range (kg) | BW Mean (kg) | CS | PK | LDH | MDH |
|---|---|---|---|---|---|---|---|
| *S. lewini* | 10 | 61.0 – 156.6 | 79.5 ± 33.9 | 1.08 ± 0.59 | 41.03 ± 11.92 | 203.19 ± 96.84 | 55.15 ± 19.09 |
| *G. cuvier* | 22 | 4.5 – 141.6 | 61.3 ± 43.3 | 0.99 ± 0.82 | 126.61 ± 71.40 | 144.53 ± 95.37 | 25.19 ± 9.51 |
| *C. plumbeus* | 24 | 7.1 – 41.5 | 27.1 ± 14.4 | 0.92 ± 0.66 | 35.05 ± 26.35 | 31.80 ± 24.09 | 16.75 ± 6.37 |
| *C. limbatus* | 5 | 9.9 – 49.1 | 22.0 ± 15.5 | 0.44 ± 0.09 | 23.85 ± 13.08 | 84.64 ± 20.43 | 19.02 ± 9.78 |
| *H. griseus* | 9 | 54.7 – 341.6 | 142.5 ± 83.8 | 0.56 ± 0.32 | 26.55 ± 15.39 | 22.14 ± 10.49 | 10.49 ± 6.98 |
| *E. cookei* | 5 | – | – | 0.52 ± 0.34 | 68.05 ± 50.79 | 141.55 ± 44.86 | 14.49 ± 5.88 |

Body weights (BW) were estimated using published length-weight regressions for each species.

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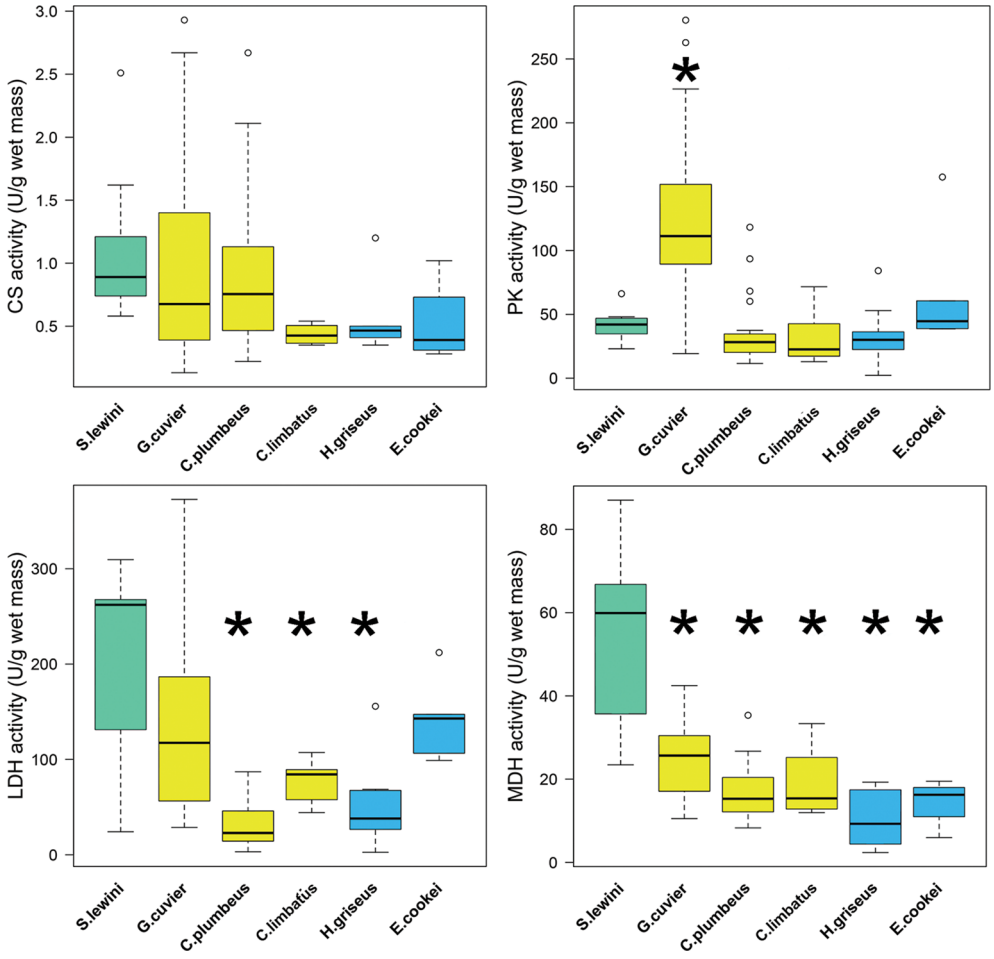


FIGURE 2
Boxplots of white muscle CS, PK, LDH, and MDH activities (U $g^{-1}$ wet mass at 10°C) from (left to right) *Sphyrna lewini* (teal), *three* coastal species (yellow) *(Galeocerdo cuvier*, *Carcharhinus plumbeus*, *Carcharhinus limbatus*), and two deep-water species (blue) (*Hexanchus griseus*, *Echinorhinus cookei*). Thick line in each represents the mean, box ends are the 25$^{th}$ and 75$^{th}$ quantiles, dashed lines are min and max values, circles are outliers. Asterisks (*) indicate significant differences in species means in comparison to *S. lewini* (*P*-values < 0.05, Welsh's ANOVA *post-hoc* Games-Howell test).

conditions. MDH plays a key role in the malate-aspartate cycle, to shuttle electrons across the inner mitochondrial membrane after the conversion of lactate to pyruvate (Ombres et al., 2011). The high MDH activity measured in *S. lewini*, in comparison to all other species examined, suggests a higher potential for this process in the white muscle under aerobic conditions (Suarez et al., 1985; Ombres et al., 2011). This is further supported by the high MDH: CS ratio observed in *S. lewini* (Table 2, Figure 3B). CS activity often scales with whole-body metabolic rate (Childress and Somero, 1979; Dahlhoff, 2004; Drazen and Seibel, 2007). Tiger (*G. cuvier*) and sandbar (*C. plumbeus*) sharks also have high CS activities, but not nearly the correspondingly high MDH activity measured in *S. lewini*, as reflected in the MDH: CS ratios (Figure 3B). MDH and CS are part of the citric acid cycle, and their activities in a given tissue should co-vary with mitochondrial content if their only role is as catalysts in the primary aerobic metabolic pathway (Dalziel et al., 2017). Thus, finding that MDH and the MDH: CS ratio are higher in *S. lewini* than in the other species studied suggests a more important role for MDH in *S. lewini*. The predominant role is likely to facilitate lactate clearance for post-exercise recovery after dives, but it may also be involved in redox balance during high-intensity activity. Although challenging, future studies measuring the production of lactate during dives and its subsequent fate during recovery from intensive activity, would be needed to distinguish

TABLE 2 Ratios (mean ± standard deviation) of MDH to CS enzyme activity in white muscle of each species.

| Species | N | MDH/CS |
|---|---|---|
| *S. lewini* | 10 | 57.1 ± 22.9 |
| *G. cuvier* | 20 | 39.5 ± 26.2 |
| *C. plumbeus* | 22 | 24.8 ± 23.2 |
| *C. limbatus* | 5 | 48.4 ± 24.9 |
| *H. griseus* | 7 | 24.7 ± 20.2 |
| *E. cookei* | 3 | 24.8 ± 11.2 |

Statistical comparisons of MDH: CS ratios between *S. lewini* and all other species except *E. cookei* were not significant (all *P*>0.05).

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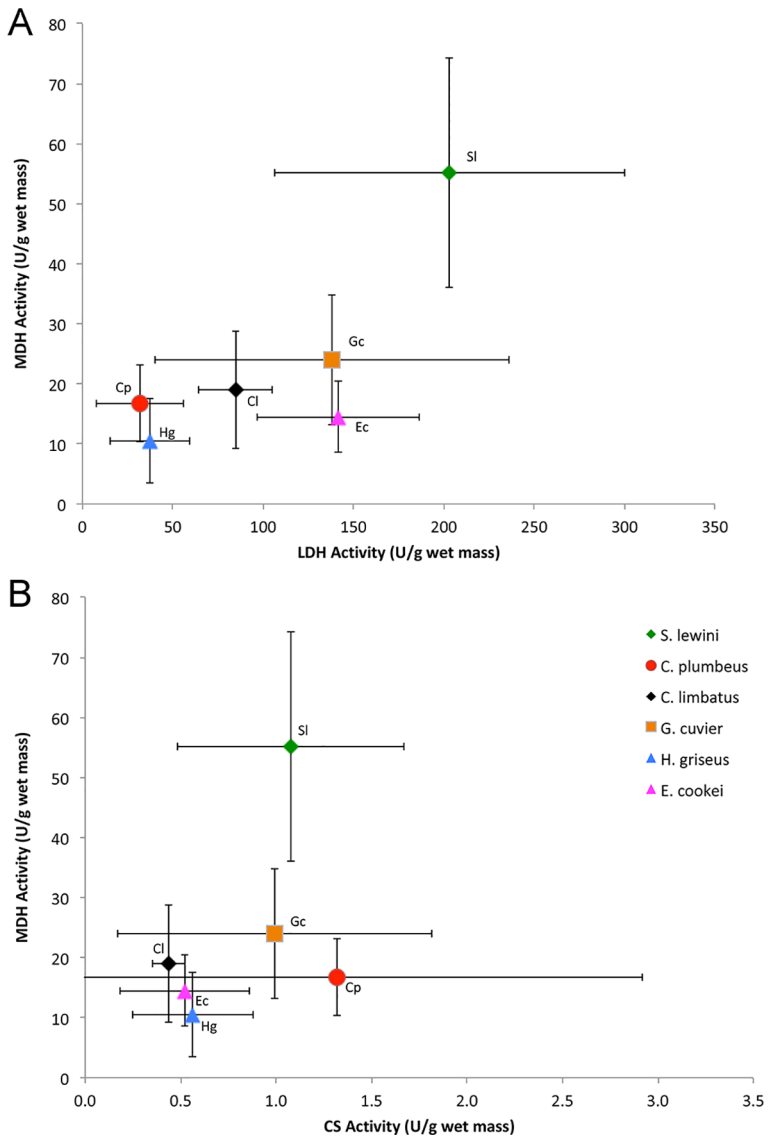


FIGURE 3
Comparison of mean white muscle activities (U $g^{-1}$ wet mass at 10°C) of **(A)** LDH versus MDH and **(B)** CS versus MDH in the six shark species studied. Error bars indicate standard deviations. Though the statistical comparisons of MHD: CS ratios between *S. lewini* and all other species except *E. cookei* were not significant (all $P>0.05$), it is noticeable that *G. cuvier* and *C. plumbeus* also have high CS activity but not the correspondingly higher MDH activity observed in *S. lewini*.

among these alternatives and fully understand which biochemical processes underly the diving and foraging behavior of *S. lewini*.

Lactate processing may be initiated when *S. lewini* reaches the "inflection point" of the dive ascent at ~250 m, which marks reduced swimming activity and a slower rate of ascent, associated with rapidly cooling muscle temperatures (Meekan and Gleiss, 2023; Royer et al., 2023). Available evidence suggests that this is the moment when *S. lewini* resumes ram-ventilation in adequately oxygenated waters, which would allow rapid repayment of any oxygen debt incurred and to remove lactate accumulated during the dive (Meekan and Gleiss, 2023; Royer et al., 2023). Converting lactate into glycogen via gluconeogenesis *in situ* under aerobic conditions during the inter-dive period would allow *S. lewini* to reduce lactate concentration and replenish white muscle glycogen stores for subsequent deep dives (Iosilevskii et al., 2022). Gluconeogenesis could also occur in other tissues such as the liver and kidney (Suarez et al., 1985). Future studies should investigate the activities of key enzymes in the gluconeogenesis pathway in multiple tissues of *S. lewini* and other sharks. Although evidence for gluconeogenesis in white muscle has been observed in several teleosts (Milligan and Wood, 1986; Girard and Milligan, 1992), a study of activities of several key gluconeogenic enzymes in the white and red locomotor muscle, liver, and heart in mako, blue, and leopard sharks found no evidence for gluconeogenic capacity (Backey, 2007). Nevertheless, rapid removal of lactate by conversion to pyruvate and oxidation of pyruvate in the citric acid cycle would be beneficial for repeated dives by *S. lewini*.

In comparison to sympatric coastal and deep-water shark species, the higher capacity of *S. lewini* for aerobic and anaerobic

metabolism and hence high activity during deep dives may confer a competitive advantage in actively foraging for deep-dwelling prey which may be relatively sluggish due to low body temperatures and metabolic rates (Childress, 1995; Seibel et al., 2000; Seibel and Drazen, 2007). Previous studies revealed a significant decline in tissue anaerobic capacities of the white muscle of deep-dwelling teleosts and cephalopods with increasing minimum depths of occurrence (Childress and Somero, 1979; Sullivan and Somero, 1980; Drazen and Seibel, 2007). For the sympatric coastal species that are adapted to warm-temperate waters, the deleterious physiological effects of moving into colder water make these deep prey resources generally inaccessible. Deep-water sharks (e.g. *H. griseus* and *E. cookei*) on the other hand are able to permanently inhabit these cold depths with access to deep prey patches, with the trade-off of living with reduced metabolic capacity. In contrast, by maintaining a high capacity for burst swimming and briefly maintaining a warm body temperature (Royer et al., 2023), *S. lewini* are able to conduct their intensive deep-dives to exploit an ecological niche that is inaccessible to sympatric coastal species (e.g., coastal carcharhinids), and have a more competitive, albeit transient, hunting capacity than the shark species that permanently inhabit these deep depths. The tradeoff for *S. lewini* is that they must have the energy reserves needed to power these brief and intensive deep dives (Meekan and Gleiss, 2023). Another potential trade-off is the increased vulnerability to predation during the inter-dive recovery period, similarly to beaked whales after their deep dives (Aguilar de Soto et al., 2020; Siegal et al., 2022).

Important caveats must be considered when interpreting the results of this study. Although it is known that enzyme activity scales with body size in many fishes, we did not detect any scaling effects in our study, most likely due to low sample sizes and limited size ranges for each species. Enzymatic activity can change based on a number of factors such as sampling period, time of day, recent feeding or mating events, and fish activity level (Yang and Somero, 1993; Dahlhoff, 2004; Li et al., 2012) - none of which are known for any of the sharks that were sampled for this study, but could explain some of the variability in the data. However, the same factors affect other published studies of fish samples from wild populations. Tissue samples were collected from live sharks under challenging field conditions and with rapid handling times to ensure proper animal welfare upon release, especially for species that are more sensitive to capture stress, particularly *S. lewini* (Hutchinson et al., 2023).

## Conclusions

The scalloped hammerhead shark possesses enzyme characteristics that probably facilitate anaerobic metabolism during deep dives and rapid recovery during inter-dive intervals through the rapid aerobic breakdown of anaerobic end products. The high aerobic and anaerobic metabolic capacities measured in the white muscle of *S. lewini* are likely crucial for conducting repeated high-activity, deep dives while the shark "holds its breath" by suppressing gill function. High levels of MDH likely facilitate clearance of lactate and possibly restoration of glycogen stores in the white muscle when the sharks are in well-oxygenated surface waters. The results also raise further questions about the oxygen storage capacity of *S. lewini*, and whether hypoxia occurs during deep foraging dives (Meekan and Gleiss, 2023). A greater understanding of the metabolic pathways and oxygen demands during dives and post-dive recovery may explain the paradox of how this species is capable of routinely exerting itself to conduct intense deep dives while suppressing gill function, yet is also highly vulnerable to rapid stress-induced mortality from fishing capture. This ability to forage actively in deep waters and to rapidly recover from periods powered by anaerobic metabolism may be key to the ecological success of *S. lewini*, which has historically been circumglobally abundant in coastal and offshore waters (Rigby et al., 2019).

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The animal study was approved by Shark capture and sampling activities were carried out in accordance with the animal use protocols of the University of Hawai'i Institutional Animal Care and Use Committee (IACUC) and were approved under IACUC protocols #05-053 and #11-1242. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

MR: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. DG: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing – review & editing. KD: Conceptualization, Data curation, Formal Analysis, Methodology, Resources, Software, Supervision, Validation, Writing – review & editing. KW: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. CM: Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. KH: Funding acquisition, Resources, Supervision, Writing – review & editing, Validation. JD: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Author disclaimer

The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subdivisions.

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