Journal of Experimental Biology and Agricultural Sciences, August - 2023; Volume – 11(4) page 683 – 695

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Acclimation to warm temperatures modulates lactate and malate dehydrogenase isozymes in juvenile *Horabagrus brachysoma* (Günther)

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Received – May 01, 2023; Revision – July 24, 2023; Accepted – August 18, 2023
Available Online – August 31, 2023

DOI: http://dx.doi.org/10.18006/2023.11(4).683.695

KEYWORDS

Horabagrus brachysoma

Acclimation temperature

LDH

sMDH

Zymography

ABSTRACT

Differential expression of isozymes enables fish to tolerate temperature fluctuations in their environment. The present study explores the modulation of lactate dehydrogenase (LDH) and cytoplasmic malate dehydrogenase (sMDH) isozyme expression in the heart, muscle, brain, liver, gill, and kidney of juvenile *Horabagrus brachysoma* after 30 days of acclimation at 26, 31, 33, and 36°C. LDH and sMDH zymography were performed using native polyacrylamide gel electrophoresis. The zymography revealed five distinct bands of LDH isoenzymes (labelled from cathode to anode as LDH-A_4, LDH-A_3B_1, LDH-A_2B_2, LDH-A_1B_3, and LDH-B_4) and three distinct bands of sMDH isoenzymes (labelled from cathode to anode as sMDH-A_2, sMDH-AB, and sMDH-B_2), with considerable variation in their expression in the tissues. Acclimation to the test temperatures did not influence the expression patterns of LDH or sMDH isozymes. Densitometric analysis of individual isozyme bands revealed a reduction in the densities of bands containing the LDH-B and sMDH-B molecules, while the densities of bands containing the LDH-A and sMDH-A molecules increased in the gills and muscle, indicating the role of these organs in adaptive responses to thermal acclimation. However, the total densities of the LDH and sMDH isozymes increased with higher acclimation temperatures, indicating that adaptation to increased temperatures in *H. brachysoma* is primarily characterised by quantitative changes in isozyme expression.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Most fish species being poikilothermic animals, fluctuations in the water temperature profoundly influence their metabolism, behaviour, migration, growth, reproduction, and ultimately survival. However, every fish species has a unique adaptive capacity, both behavioural and physiological, to endure the temperature change in their environment. Their physiology is modulated in a variety of ways, with the rise and fall in temperature extending the thermal tolerance range of the species (Ficke et al. 2007; McKenzie et al. 2021). The biochemical mechanism for temperature adaptations in fish involves modulations in the activities of metabolic enzymes, the lipid composition of cellular membranes, quantitative changes in total or specific protein in different organs, and the manifestation of isozymes that allow for continuous functions under altered temperature conditions (Seddon 1997; Morgan et al. 2022). Most enzymes that play an important role in adaptive response in fish are linked to energy-producing pathways, such as glycolysis, gluconeogenesis, pentose phosphate pathway, Kreb's cycle, respiratory chain, digestion, and protein metabolism (Guillen et al. 2019; Volkoff and Rønnestad 2020; Li et al. 2023). Organisms often produce isozymes that allow them to adapt and function at altered environmental temperatures (Tattersall et al. 2012; Sejian et al. 2018). The adaptive responses to thermal acclimation involve both qualitative and quantitative changes in the metabolic enzymes. Quantitative changes involve the production of varying quantities of an enzyme, while qualitative changes involve the kinds of enzymes that are produced (Schulte 2004; Tattersall et al. 2012). The outcome of these adaptative processes is to maintain a constant metabolic function at altered environmental temperatures.

Fish acclimated to warm and cold temperatures may differ in the rates of their gene expression, resulting in different amounts of an enzyme being synthesized (Schulte 2004; Badr et al. 2023). The channel catfish (*Ictalurus punctatus*) after cold acclimation showed a two-fold increase in its liver weight, size of cell, total protein, and activity of enzymes including cytochrome oxidase, lactate dehydrogenase, citrate synthase, glucose-6-phosphate dehydrogenase (G6PDH), and 6-phosphogluconate dehydrogenase (Kent et al. 1988). Similarly, quantitative changes in liver G6PDH were detected by immunodetection technique during cold acclimation in *I. punctatus* (Seddon 1997). An alternative to the quantitative theory is the synthesis of qualitatively distinct molecules, in which the warm and cold-acclimated fish would differ mainly in the proportion of the genotype being expressed, such that the populations express isozymes that are qualitatively different (Schulte 2004; Luo et al. 2022). A direct correlation between isozyme expression and temperature acclimation was reported in the brain acetylcholinesterase (AChE) of the trout (*Salmo gairdnerii*) with two variants, namely warm-variant and cold-variant, of which only the warm-variant occurred during warm acclimation (17°C) and the cold-variant occurred only during cold acclimation (2°C), while both isozymes (variants) were present during acclimation at an intermediate temperature (12°C) (Baldwin and Hochachka 1970). In contrast, no changes were reported in the level of isozymes in *Gillichthys mirabilis*, *Ameiurus nebulosus*, *Gibbonsia elagans*, *G. metzi*, and *G.montereyensis* in response to changes in environmental temperatures (Somero 1975). While the concept of on-off isozyme control, which accounts for the quantitative and qualitative changes, is intriguing, another plausible hypothesis of temperature-dependent conformational changes in the enzymes has been suggested (Poly 1997; Aswani and Trabucco 2019). Studies on the orthologs of LDH have shown the importance of pH and osmolytes (low-molecular-mass organic solutes) in maintaining the structural and functional characteristics of enzymes (Somero 2004).

Horabagrus brachysoma is an endemic catfish with a natural range in the rivers of Kerala (Ali et al. 2007), Karnataka (Bhat 2001), and the Northern Western Ghats in Maharashtra (Katwate et al. 2012). The catfish is propagated for small-scale aquaculture (Raghavan et al. 2016) and is valued for the ornamental fish trade (Ali et al. 2007; Sureshkumar 2013). In earlier research on *H. brachysoma*, it was reported that acclimation to increasing temperatures from 15 to 36 ℃ increased the heat tolerance and metabolic rates, with optimal physiological performance between 31 and 33 °C (Dalvi et al. 2009); the correlation of its elevated heat tolerance with the elevated HSP70 after acclimation at 20 and 30 °C (Dalvi et al. 2012); and the modulation of metabolic enzymes and HSP70 with increasing temperatures (Dalvi et al. 2017).

With the escalating concerns about global warming and its effect on ecosystems, research is now focused on understanding the molecular mechanisms that help organisms adapt to changing climatic conditions (McCaw et al. 2020; Aubry and Williams 2022). A vast literature documents the efforts that have been made to understand such adaptive responses. However, there is a dearth of information on the adaptive mechanisms of fish endemic to India. The current study was therefore undertaken to understand the modulation of lactate dehydrogenase (LDH) and cytoplasmic malate dehydrogenase (sMDH) isozymes for thermal adaptation in *H. brachysoma* acclimated at 26, 31, 33, and 36°C.

2 Materials and methods

2.1 Experimental fish and experimental setup

Juveniles of *H. brachysoma* (Average length 10.35 ± 2.78 cm) were procured from Aquatic World, Mumbai, India, and transported live with proper aeration to the wet laboratory of the Central Institute of Fisheries Education, Mumbai, India. The fish were given a prophylactic dip in salt solution (2%) and then acclimatized to laboratory conditions (26 ± 1 °C) for 30 days,

during which the fish were daily fed with live *tubifex* worms *ad libitum*. The experimental setup consisted of 24 fish equally distributed in 4 thermostatic aquariums (6 fish per test temperature; water capacity 52 L; sensitivity ± 0.2°C) maintained at 26°C. One of the aquariums maintained at 26 °C was used as the control. Acclimation to elevated temperatures of 31, 33, and 36°C and maintenance of the fish were done as described earlier by Dalvi et al. (2009), and Dalvi et al. (2017). After 30 days of acclimation, the fish were fasted for 24 hours before sampling.

2.2 Sample preparation and protein estimation

Following 30 days of acclimation, fish from the test temperatures were anaesthetized with 50 μL^{-1} clove oil and dissected to extract the tissues, such as gill, brain, heart, kidney, liver, and muscle. The tissues were immediately washed twice with chilled phosphate buffer (50 mM, pH 7.2), blotted on filter paper, weighed, and homogenised (20% weight/volume) individually in chilled phosphate buffer (50 mM, pH 7.2, with 0.1 mM PMSF, and 0.25M sucrose) using a glass homogenizer kept in an ice bath. Individual tissue homogenates were centrifuged for 20 minutes at 10,000 rpm at 4°C, the supernatants of each tissue homogenate were collected separately in a glass vial and kept at -20°C until use. The protein contents in each supernatant were determined using the Bradford (1976) method with bovine serum albumin as a standard.

2.3 Electrophoresis and zymography

Lactate dehydrogenase (LDH) and cytoplasmic malate dehydrogenase (sMDH) isozymes in various tissues were resolved using non-denaturing polyacrylamide gel electrophoresis (ND-PAGE) in a vertical slab gel electrophoresis apparatus (Microkin, TechnoSource, Mumbai, India) with a discontinuous system following the method of Walker (2002). The LDH isozymes were resolved using ND-PAGE with 5% stacking and 10% separating gels, while the sMDH isozymes were resolved using ND-PAGE with 5% stacking and 12% separating gels. Since the concentration of LDH and sMDH varied in different tissues, the amount of protein to be loaded on the PAGE for good resolution of the isozymes was different for each tissue (Figures 2 and 5). However, aliquots of the supernatant with equal protein concentrations were loaded on the gel for each tissue to be tested at different acclimation temperatures. Two to three replicates of each tissue from different test temperatures were electrophoresed simultaneously on the ND-PAGE with a constant voltage (50V) for 3 hrs at 4°C. The LDH and sMDH isozymes were visualized by activity staining in the gel following the methods described by Pasteur et al. (1988), with modifications. Briefly, the LDH gels were incubated in a solution containing 0.5 M Tris-HCl buffer (pH 7.5), NAD (3%), NBT (0.4%), PMS (0.01%), and sodium lactate (0.2 N). Similarly, the sMDH gels were incubated in a solution of 0.5 M Tris-HCl buffer (pH 7.5), NAD (3%), NBT (0.4%), PMS (0.01%), and malic acid (0.2 N). The gels were incubated for 30 minutes at 30°C. Human serum was loaded into the gels for LDH isozyme for comparison and standardization of the procedure. No artifacts were observed in the gels that were stained in buffer without the substrates for LDH and sMDH. The stained gels were rinsed with distilled water and images were captured using a gel documentation system (Syngene, UK). The LDH and sMDH isoenzymes were nomenclated following Shaklee et al. (1990). The absolute integrated optical density (IOD) of each isoenzyme band was quantified using the Gel-pro analyzer (version 4.5, Media Cybernetics, USA) and represented as area density per µg protein. The densities of all isoenzyme bands in a lane for each tissue tested were summed to investigate the change in the total activities of the enzymes at different acclimation temperatures.

2.4 Statistical analysis

The area densities of each isozyme type and the summed densities of the isoenzymes for an organ of fish acclimated to the four experimental temperatures were compared via one-way ANOVA using Statistical Package for the Social Sciences (version 16.0, USA). Duncan's multiple range tests were used to determine the differences among treatment means at $P<0.05$. The data presented in the figures are expressed as the mean ± standard error of the mean (SEM).

3 Results

3.1 Lactate dehydrogenase (LDH)

Five distinct LDH isoenzymes were observed in *H. brachysoma*, with considerable variation in their expression patterns in different tissues (Figure 1). The LDH isoenzymes were nomenclated as LDH-A_4, LDH-A_3B_1, LDH-A_2B_2, LDH-A_1B_3, and LDH-B_4 based on their relative electrophoretic mobility. A distinct anodal band (LDH-B_4) was observed in the liver, and a single cathodal band (LDH-A_4) was observed in the muscle. However, in the liver, electrophoresis with higher protein concentrations (5 or 10µg) revealed three faintly stained heterotetramers (LDH-A_3B_1, LDH-A_2B_2, and LDH-A_1B_3) in addition to the homotetramer of LDH-B (LDH-B_4), while in the muscle, the heterotetramers were not detected at higher protein concentrations (5 or 10µg) (Data not shown). The heart, brain, gills, and kidney showed the presence of all five isozymes, consisting of homotetramers and heterotetramers of the LDH-A and LDH-B subunits, but the IODs of each isoenzyme differed for each tissue. The LDH-C isoenzyme was not detected in any of the tissues investigated.

When a specific tissue between fish acclimated at 26, 31, 33, and 36°C was compared, although the LDH isozyme expression patterns remained consistent (Figure 2), the IODs of each LDH isozyme type showed variations (Figure 3). The IODs of all the LDH isozymes

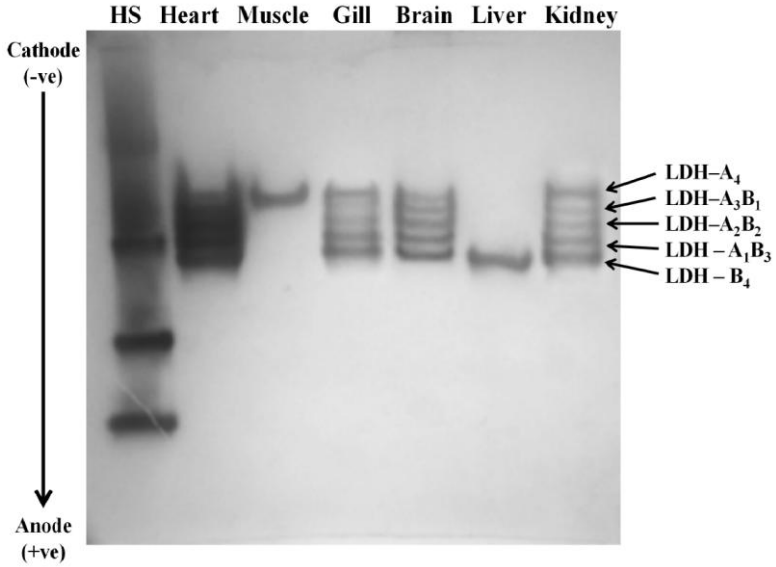

Figure 1 Comparison of lactate dehydrogenase isozyme expression in heart, skeletal muscle, gill, brain, liver, and kidney tissues of *H. brachysoma* acclimated at 26°C.The amount of protein loaded on the gel for each tissue were, heart: 2 µg, skeletal muscle: 2 µg, gill: 10 µg, brain: 5 µg, liver: 2µg, and kidney: 5 µg. The tetrameric lactate dehydrogenase represented by five isozymes are labelled from cathode to anode as LDH-A_4, LDH-A_3B_1, LDH-A_2B_2, LDH-A_1B_3, and LDH-B_4. Human serum (HS) was loaded in the first well for comparison and standardization of the procedure.

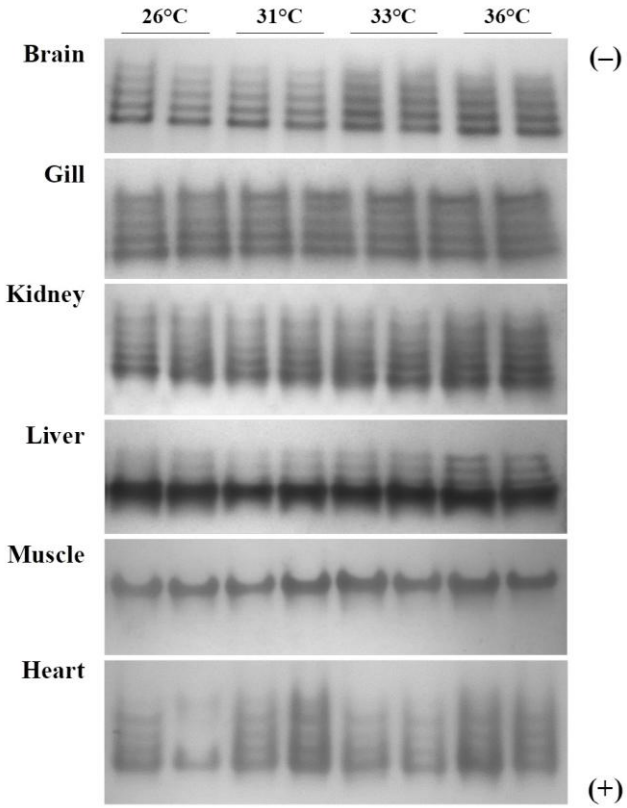

Figure 2 Comparison of lactate dehydrogenase isozyme expression in brain, gill, kidney, liver,muscle, and heart tissues of *H. brachysoma* acclimated to the test temperatures. The amount of protein loaded on the gel for each tissue were, brain- 5 µg, gill- 10 µg, kidney- 5 µg, liver- 5 µg, skeletal muscle- 2µg, and heart- 2 µg.

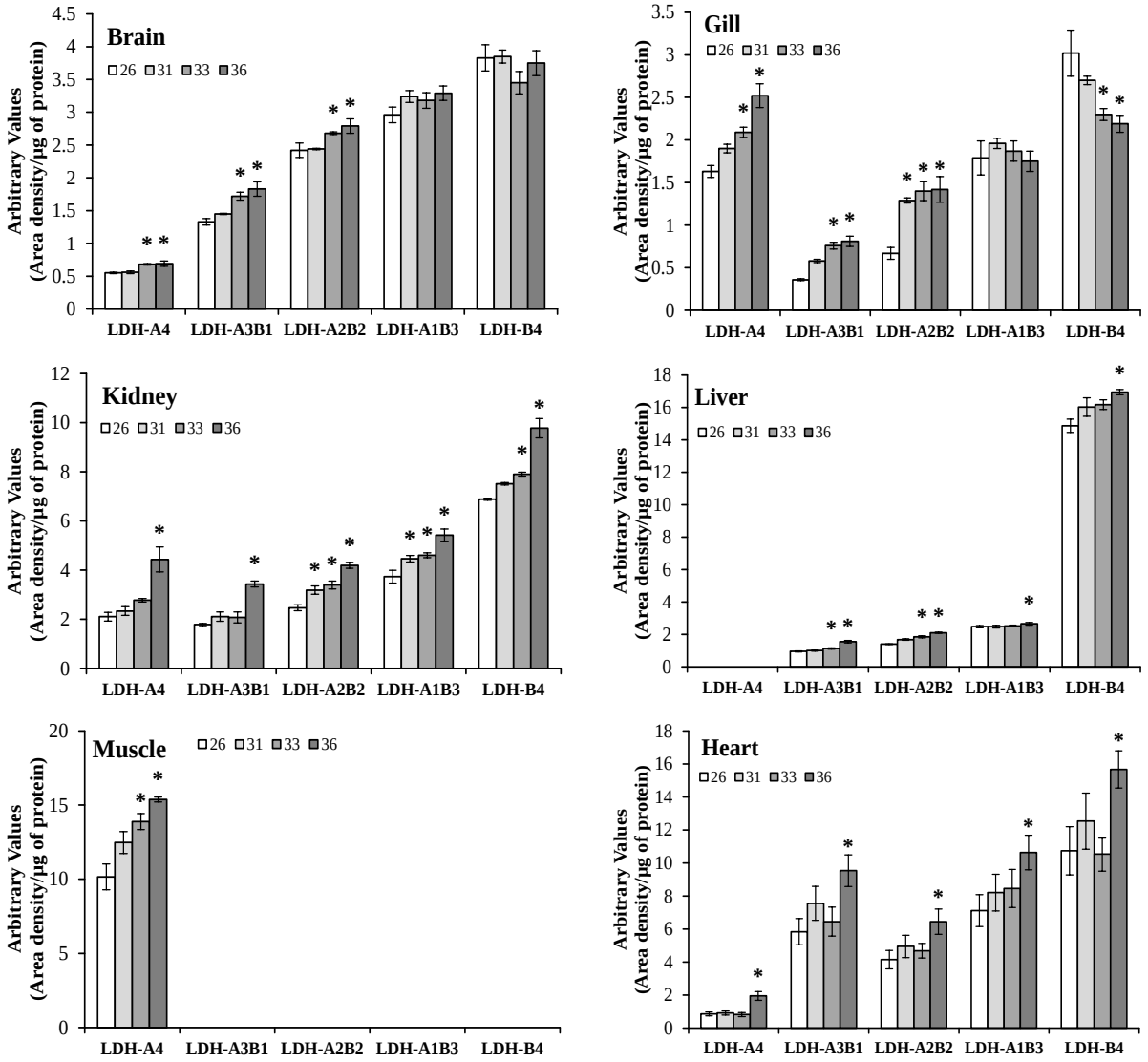

Figure 3 Area densities (IODs) of lactate dehydrogenase isozyme in in brain, gill, kidney, liver,muscle, and heart tissues of *H. brachysoma* acclimated to the test temperatures. Value expressed as area density/µg of protein. Data represents mean ± SE, n=6. The bars with the asterisk '*' indicate significant (P<0.05) differences among the test temperatures.

significantly increased (P<0.05) in the kidney, liver, muscle, and heart tissues at either 33 and/or 36°C. In the brain, the IODs of LDH-A_4, LDH-A_3B_1, and LDH-A_2B_2 significantly (P<0.05) increased at 33 and 36°C than at lower temperatures. In the gills, the IODs of LDH-A_4, LDH-A_3B_1, and LDH-A_2B_2 significantly (P<0.05) increased, while the IOD of LDH-B_4 significantly (P<0.05) decreased at 33 and 36°C. A significant increase (P<0.05) was also observed in the LDH-A_2B_2 and LDH-A_1B_3 in the kidney and the LDH-A_2B_2 in the gill at 31°C and higher temperatures. The total densities (sum of IODs) of the LDH isozyme significantly (P<0.05) increased in the kidney at 31°C and higher acclimation temperatures, in the liver and muscle at 33 and 36°C, and in the heart at 36°C (Figure 7A).

3.2 Cytoplasmic malate dehydrogenase (sMDH)

Three distinct sMDH isoenzymes were observed in different tissues of *H. brachysoma*, with considerable variation in their expression patterns (Figure 4). The MDH is a multimeric enzyme made up of dimers or tetramers of subunits with molecular weights ranging from 30 to 35 kDa (Goward and Nicholls 1994). The sMDH isozymes were nomenclated from the cathode to the anode as sMDH-A_2, sMDH-AB, and sMDH-B_2. The muscle and heart predominantly expressed sMDH-AB and sMDH-B_2, while the liver expressed sMDH-A_2 and sMDH-AB isozymes. Although all three sMDH isozymes were expressed in the gill, brain, and kidney, considerable differences were observed in their IODs in the selected tissues.

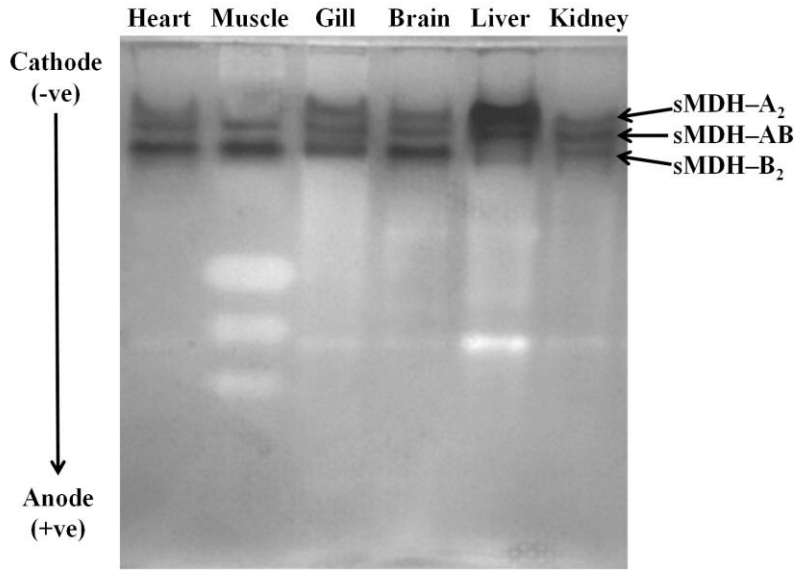

Figure 4 Comparison of cytoplasmic malate dehydrogenase isozyme expression in heart, skeletal muscle, gill, brain, liver, and kidney tissues of *H. brachysoma* acclimated at 26°C. The dimeric matate dehydrogenase represented by three isozymes are labelled from cathode to anode as sMDH-A_2, sMDH-AB, and sMDH-B_2. The amount of protein loaded on the gel for each tissue were, heart- 10 µg, skeletal muscle- 10 µg, gill- 20 µg, brain- 10 µg, liver- 5 µg, and kidney- 10 µg.

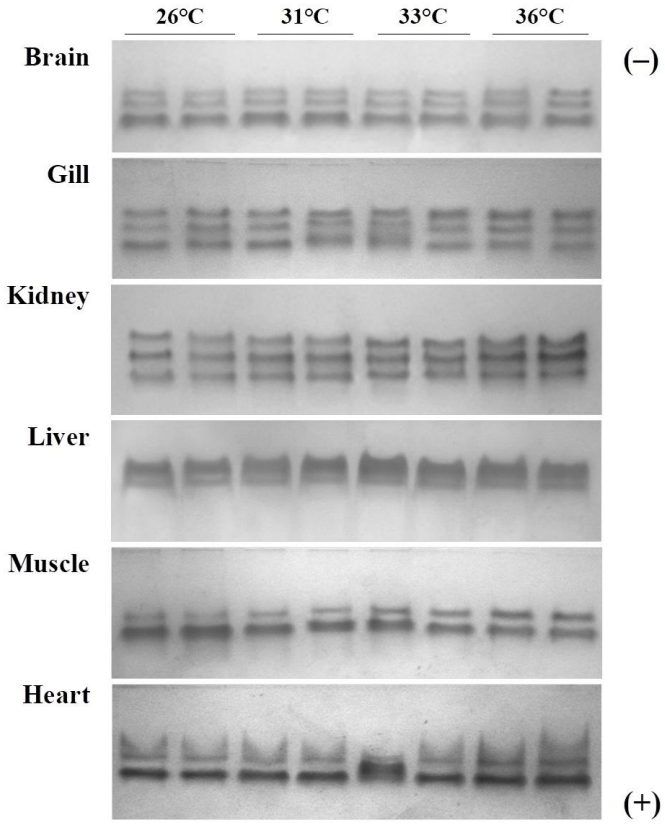

Figure 5 Comparison of cytoplasmic malate dehydrogenase isozyme expression in brain, gill, kidney, liver, muscle, and heart tissues of *H. brachysoma* acclimated to the test temperatures.The amount of protein loaded on the gel for each tissue were, brain- 10 µg, gill- 20 µg, kidney- 10 µg, liver- 5 µg, skeletal muscle- 10 µg, and heart- 10 µg.

When a specific tissue between fish acclimated at 26, 31, 33, and 36°C was compared, although the sMDH isozyme expression patterns remained consistent (Figure 5), the IODs of each MDH isozyme type showed variations (Figure 6). The sMDH-A_2 significantly (P<0.05) increased in the gills, kidney, and liver tissues, while the sMDH-AB significantly (P<0.05) increased in the gills, kidney, muscle, and heart tissues either at 33 and/or 36°C. However, in the muscle, the sMDH-B_2 significantly decreased (P<0.05) in fish acclimated at 36°C. The total densities (sum of IODs) of the sMDH isozymes significantly increased (P<0.05) in the gills, kidney, liver, and heart at 33 and/or 36°C, but not in the brain and muscle tissues (Figure 7B).

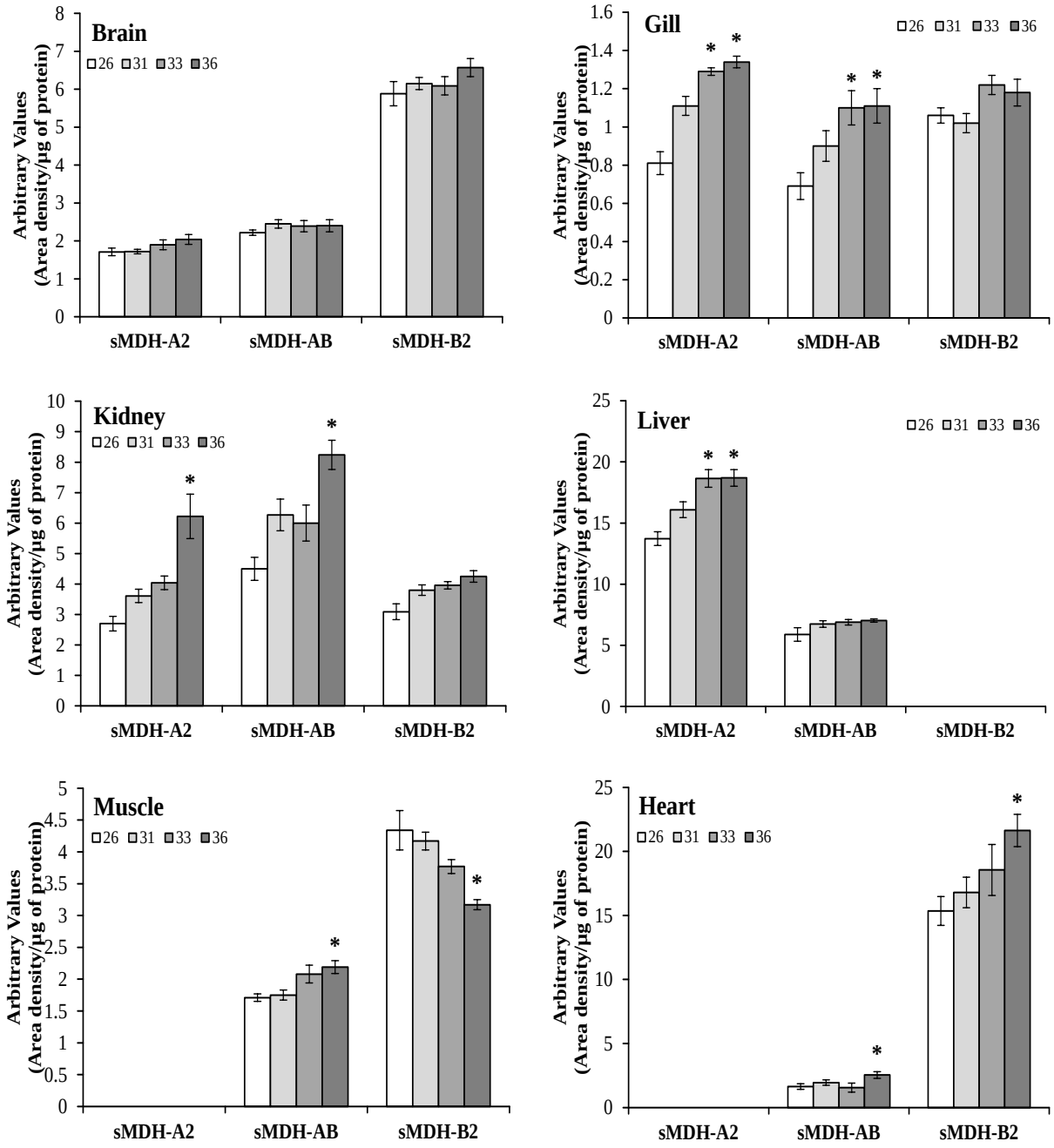

Figure 6 Area densities (IODs) of cytoplasmic malate dehydrogenase isozyme in brain, gill, kidney, liver,muscle, and heart tissues of *H. brachysoma* acclimated to the test temperatures. Value expressed as area density/µg of protein. Data represents mean ± SE, n=6. The bars with the asterisk '*' indicate significant (P<0.05) differences among the test temperatures.

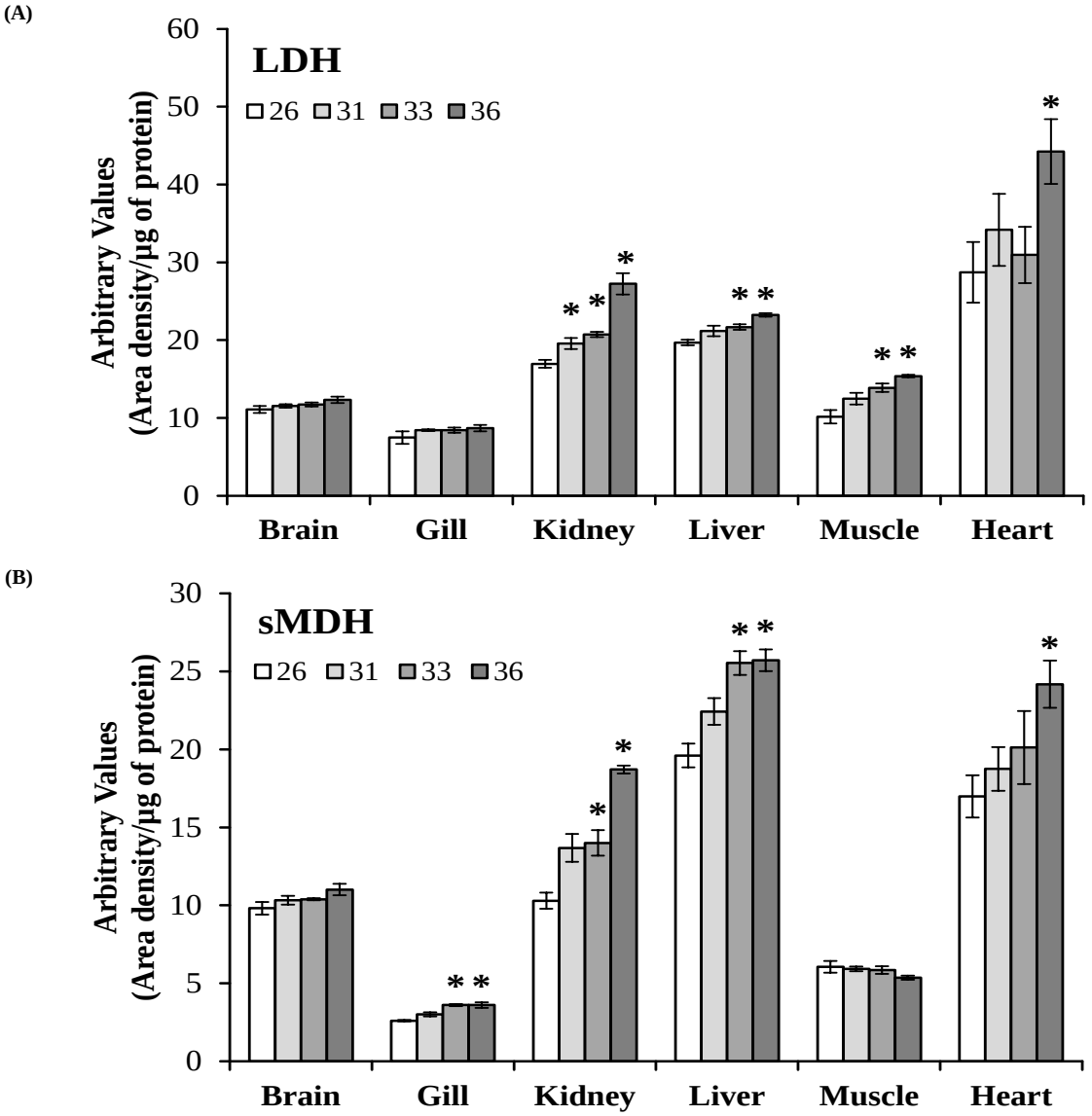

Figure 7 Summed area densities (IODs) of lactate dehydrogenase (A) and cytoplasmic malate dehydrogenase (B) isozyme expressed in brain, gill, kidney, liver, muscle, and heart tissues of *H. brachysoma* acclimated to the test temperatures. Value expressed as area density/µg of protein. Data represents mean ± SE, n=6. The bars with the asterisk '*' indicate significant (P<0.05) differences among the test temperatures.

4 Discussion

LDH is the most widely researched enzyme in fish. It is a cytosolic enzyme that catalyses the interconversion of lactate to pyruvate in the final step of anaerobic glycolysis and also the conversion of lactate to glucose during gluconeogenesis (Zakhartsev et al. 2004; Koenekoop and Åqvist 2023). It is a tetrameric protein that is controlled by three distinct genes viz., LDH-A*, LDH-B*, and LDH-C*. The random combination of these gene products results in the formation of different isoenzymes that are known to have a tissue-specific distribution. In fish, the LDH-A is chiefly expressed in tissues with anaerobic glycolysis, like the skeletal muscle; the LDH-B is chiefly expressed in tissues with aerobic metabolism, such as the heart and/or liver; and the LDH-C is typically expressed in the liver or eyes, often co-occurring with the LDH-B (de Almeida-Val and Val 1993; Ahmad 2009). In the present study, the normal (not reverse) electrophoretic mobility of the LDH-A and LDH-B subunits in *H. brachysoma* is similar to those reported earlier in most teleost fishes (Markert and Faulhaber 1965; Rao et al. 1989; Ferreira et al. 1991; El-alfy et al. 2008). Differences observed in the expression patterns of LDH isozymes in the selected tissues of *H. brachysoma* (Figure 1) indicate restricted or preferential subunit assembly as an adaptation strategy to meet metabolic demands (aerobic or anaerobic) (Coquelle et al.

2007) or restricted expression of the subunit in the tissue (Murphy et al. 1990; Ahmad 2009). Our observations on the expression patterns of the LDH-A and LDH-B subunits in different tissues are similar to those in the catfishes *Clarias batrachus* and *Heteropneustes fossilis* (Triveni and Rao 1986) and the murrel *Channa punctata* (Ahmad 2009). The LDH-C gene is predominantly expressed in the liver or the eyes of most teleost fishes (Rao et al. 1989; Powers and Schulte 1998; Schulte 2004). It is noteworthy that in the present study, the LDH-C was not detected in liver tissue.

In this study, the expression patterns of LDH (Figure 2) and sMDH (Figure 5) isozymes were consistent in *H. brachysoma* acclimated to increasing temperatures. The findings of this study are similar to those reported for LDH in the loach *Misgurnus fossilis* (Ozernyuk et al. 1994) and crucian carp *Carassius carassius* (Vornanen 1994) during seasonal temperature changes and in the cod *Gadus morhua* acclimated to 4°C and 12°C (Zakhartsev et al. 2004). Recently, Luo et al. (2022) showed that exposure to cold (13°C) or heat (23°C) shock did not influence the muscle LDH isozyme expression pattern in Amur sturgeon (*Acipenser schrenckii*). Aswani and Trabucco (2019) observed that the two isozymes of brain AChE (a 59 Kda monomer and a 244 Kda tetramer) in *Tilapia mossambica* acclimated to 25 and 37°C had similar electrophoretic mobility and intensity but differed in their Kms, thermal stability, and activation energy, and suggested that the temperature adaptation of brain AChE in *T. mossambica* involves the aggregation-dissociation of the two isozymes. However, in the present study, the sum densities of the isozymes in different tissues increased at either 33 and/or 36°C, indicating enhanced expression of their respective genes at higher temperatures. In the case of LDH, such a phenomenon may indicate temperature-induced metabolic reorganization towards aerobic or anaerobic metabolism at warm temperatures (Somero 1973; Lannig et al. 2003). Thus, the higher LDH densities (or activities) detected in all tissues of *H. brachysoma* acclimated to 36°C imply that the fish were in an anaerobic state of metabolism at this temperature, as observed in our earlier study (Dalvi et al. 2017). An earlier study on LDH in different tissues of goldfish (*Carassius auratus*) revealed increased expression of specific LDH isoforms during cold acclimation, indicating that metabolic adjustments can be achieved, at least partially, by modifying protein concentration (Hochachka 1965). Quantitative changes in the muscle LDH and brain sMDH, as determined by staining intensities in the gel, were reported in *Lepomis cynellus* acclimated at 5 and 25°C (Wilson et al. 1975). Similarly, acclimation to cold temperatures resulted in a reduction in the specific concentration of LDH in cod (*G. morhua*) liver and white muscle (Zakhartsev et al. 2004). However, contrary to our results, exposure of Amur sturgeon (*A. schrenckii*) to heat shock (23°C) decreased the intensity of a specific LDH-2 band during the 72-hour exposure period (Luo et al. 2022), indicating a species-specific response to thermal stress.

In the present study, the IODs of LDH-A_4 increased in all tissues with increasing acclimation temperatures, except for the liver. The tissue-specific distributions of LDH isozymes indicate functional divergence, as LDH-A is ideally suited for pyruvate reduction and LDH-B and LDH-C are ideally suited for lactate oxidation (Schulte 2004). Since LDH-A_4 is predominantly responsible for the conversion of pyruvate to lactate, i.e., anaerobic glycolysis, and LDH-B_4 is responsible for the conversion of lactate to pyruvate, i.e., gluconeogenesis and aerobic metabolism, it is suggested that different LDH isozymes have different functional significance (Powers and Schulte 1998). Studies on gene coding loci for LDH-A_4 in *Misgurnus fossilis* acclimated to 5 and 18°C reported that the qualities of total and LDH-A mRNA isolated from white skeletal muscle significantly increased after acclimation to 18°C compared to 5°C (Smirnova et al. 2002). Therefore, the results of this study may indicate increased production of LDH-A_4 and an enhanced pyruvate metabolism pathway in *H. brachysoma* acclimated to higher temperatures.

In this study, the LDH-B_4 isozyme decreased significantly ($P<0.05$) in the gills of *H. brachysoma* with increasing acclimation temperatures (Figure 6). Crawford and Powers (1989) compared two environmentally distinct populations of *Fundulus heteroclitus* and observed that the northern population (14.1°C) had twice the concentration of LDH-B_4 enzyme (EC 1.1.1.27) and its mRNA than the southern population (31.7°C). In a subsequent experiment, Segal and Crawford (1994) observed that when these two populations of *F. heteroclitus* were acclimated to 10°C and 20°C, both populations exhibited 1.3-fold higher levels of the LDH-B_4 enzyme at 10°C compared to 20°C, suggesting that the variations in 10°C acclimated fish are caused by differences in the protein stability of LDH-B allozymes between the northern and southern populations. Organs such as the heart or gill may first encounter reduced aerobic scope, oxygen deprivation, anaerobiosis, and energy failure at increasing temperatures (Ern et al. 2023). In our study, the reduction in LDH-B_4 and increase in LDH-A_4 isozymes in the gills with increasing acclimation temperatures indicate that warm temperatures induce the production of thermostable LDH-A molecules, profoundly affecting the respiratory organs. However, further investigation is required to confirm the thermostability of LDH-A in *H. brachysoma*.

In the present study, the sMDH isozyme expression pattern varied in different tissues of *H. brachysoma* (Figure 4). Results of this study are consistent with those reported for sMDH in *Leporinus friderici*, *Hoplias malabaricus*, and *Pimelodus maculatus* (Monteiro et al. 1998), *Leiostomus xanthurus* (Schwantes and Schwantes 1982a; Schwantes and Schwantes 1982b), and the

Astyanax fasciatus (De Luca et al. 1983), demonstrating variable expression of MDH-A and MDH-B subunits in different tissues. Schwantes and Schwantes (1982a, 1982b) first reported thermal adaptation in teleosts by two sMDH gene loci, with the sMDH-A* encoding a thermostable isoform and the sMDH-B* encoding a thermolabile isoform. A comparison of sMDH between eastern Pacific barracudas (*Sphyraena idiastes*, *S. argentea*, *S.ensis*, and *S.lucasana*) from different latitudes revealed that variations in the proportion of thermostable and thermolabile sMDH isozymes are crucial for temperature adaptation and that the lack of thermolabile isoforms can be a specific trait in species adapted to warm temperatures (Lin and Somero 1995a). Thermostable sMDH-A expression is reported in Sciaenids (*Micropogonias furnieri*, *Cynoscion striatus*, and *Macrodon ancylodon*) (Coppes et al. 1987), Amazon cichlid fishes (*Astronotus ocellatus*, *Cichla monoculus*, *Geophagus cffharreri*, *Cichlassoma severum*, and *Mesonauta insignis*) (Farias and Almeida-Val 1992), Eastern Pacific barracuda (*Sphyraena* spp)(Lin and Somero 1995a), *Gillichthys mirabilis* (Lin and Somero 1995b), and Characiformes and Perciformes fishes (Monteiro et al. 1998). In our study, the sMDH-A_2 isozyme increased significantly (P<0.05) in the gills, kidney, and liver tissues, and the sMDH-B_2 isozyme decreased significantly (P<0.05) in the muscle at 33 and 36°C (Figure 6), indicating differential expression of the thermostable sMDH-A sub-unit in the tissues for sustained metabolic functions at elevated temperatures.

Conclusion

In this study, the suite of LDH and sMDH isozymes expressed in various tissues remained unchanged regardless of acclimation temperatures. However, an increase in the sum density and the densities (IODs) of isozymes with LDH-A and sMDH-A sub-units was observed with increasing acclimation temperatures. Our results suggest that the strategies for thermal adaptation in *H. brachysoma* involve quantitative changes in the LDH and sMDH isozymes. Further, the reduction in the levels of the LDH-B and sMDH-B molecules and the increase in the LDH-A and sMDH-A molecules in the gill and muscle tissues indicate that the adaptive responses to thermal acclimation are governed by and restricted to these organs.

Acknowledgement

The first author was a senior research fellow in the Board of Research in Nuclear Sciences (BRNS)-funded thermal ecology project. The authors gratefully acknowledge funding from the Department of Atomic Energy, Government of India (BRNS Sanction No. 99/36/22/BRNS, Grant No. 089). The authors are grateful to Ms. Mythili Mukundharajan, Head Department of English, M.D.College, for proofreading the manuscript. The authors also thank Dr. Vinita Jain, librarian, M.D.College, for her timely help in checking the manuscript for plagiarism.

Conflicts of interest and financial disclosures

The authors state that they have no conflicts of interest.

References

Ahmad, R. (2009). Functional and adaptive significance of differentially expressed lactate dehydrogenase isoenzymes in tissues of four obligatory air-breathing *Channa* species. *Biologia*, *64*(1), 192–196. https://doi.org/10.2478/s11756-009-0017-7

Ali, P. A., Raghavan, R., & Prasad, G. (2007). Threatened fishes of the world: *Horabagrus brachysoma* (Gunther, 1864) (Bagridae). *Environmental Biology of Fishes*, *78*(3), 221. https://doi.org/10.1007/s10641-006-0022-4

Aswani, V., & Trabucco, D. (2019). Biochemical adaptation in brain acetylcholinesterase during acclimation to sub-lethal temperatures in the eurythermal fish *Tilapia mossambica*. *Scientific Reports*, *9*(1), 19762. https://doi.org/10.1038/s41598-019-56066-x

Aubry, L. M., & Williams, C. T. (2022). Vertebrate phenological plasticity: From molecular mechanisms to ecological and evolutionary implications. *Integrative and Comparative Biology*, *62*(4), 958–971. https://doi.org/10.1093/icb/icac121

Badr, A., Haverinen, J., & Vornanen, M. (2023). Tissue-specific differences and temperature-dependent changes in Na, K-ATPase of the roach (*Rutilus rutilus*). *Aquaculture*, *563*, 738963. https://doi.org/10.1016/j.aquaculture.2022.738963

Baldwin, J., &Hochachka, P. W. (1970). Functional significance of isoenzymes in thermal acclimatization. Acetylcholinesterase from trout brain. *Biochemical Journal*, *116*(5), 883–887. https://doi.org/10.1042/bj1160883

Bhat, A. (2001). A new report of Horabagrus brachysoma Jayaram, family Bagridae in Uttara Kannada District, Karnataka. *Journal of the Bombay Natural History Society*, *98*(2), 294–295.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1006/abio.1976.9999

Coppes, Z. L., Schwantes, M. L. B., & Schwantes, A. R. (1987). Adaptative features of enzymes from family Sciaenidae (Perciformes)—I. Studies on soluble malate dehydrogenase (s-MDH) and creatine kinase (CK) of fishes from the south coast of Uruguay. *Comparative Biochemistry and Physiology. Part B: Comparative Biochemistry*, *88*(1), 203–209. https://doi.org/10.1016/0305-0491(87)90101-5

Coquelle, N., Fioravanti, E., Weik, M., Vellieux, F., & Madern, D. (2007). Activity, stability and structural studies of lactate dehydrogenases adapted to extreme thermal environments. *Journal of Molecular Biology*, *374*(2), 547–562. https://doi.org/10.1016/j.jmb.2007.09.049

Crawford, D. L., & Powers, D. A. (1989). Molecular basis of evolutionary adaptation at the lactate dehydrogenase-B locus in the fish *Fundulusheteroclitus*. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(23), 9365–9369. https://doi.org/10.1073/pnas.86.23.9365

Dalvi, R. S., Pal, A. K., Tiwari, L. R., Das, T., & Baruah, K. (2009). Thermal tolerance and oxygen consumption in *Horabagrus brachysoma* (Gunther) acclimated to different temperatures. *Aquaculture*, *29*, 116–119. https://doi.org/10.1016/j.aquaculture.2009.06.034

Dalvi, R. S., Pal, A. K., Tiwari, L. R., & Baruah, K. (2012). Influence of acclimation temperature on the induction of heat-shock protein 70 in the catfish *Horabagrus brachysoma* (Günther). *Fish Physiology and Biochemistry*, *38*(4), 919–927. https://doi.org/10.1007/s10695-011-9578-9

Dalvi, R. S., Das, T., Debnath, D., Yengkokpam, S., Baruah, K., Tiwari, L. R., & Pal, A. K. (2017). Metabolic and cellular stress responses of catfish, *Horabagrus brachysoma* (Günther) acclimated to increasing temperatures. *Journal of Thermal Biology*, *65*, 32–40. https://doi.org/10.1016/j.jtherbio.2017.02.003

de Almeida-Val, V. M. F., & Val, A. L. (1993). Evolutionary trends of LDH isozymes in fishes. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *105*(1), 21–28. https://doi.org/10.1016/0305-0491(93)90164-Z

De Luca, P. H., Schwantes, M. L. B., & Schwantes, A. R. (1983). Adaptative features of ectothermic enzymes—IV. Studies on malate dehydrogenase of *Astyanax fasciatus* (Characidae) from lobo reservoir (São Carlos, São Paulo, Brasil). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *74*(2), 315–324. https://doi.org/10.1016/0305-0491(83)90019-6

El-Alfy, S. H., Abdelmordy, M. B., & Salama, M. S. (2008). Lactate dehydrogenase isozymes in tilapiine fishes (Cichlidae): Tissue expression and genetic variability patterns. In *Proceedings of the 8th International Symposium on Tilapia in Aquaculture* (pp. 181–197).

Ern, R., Andreassen, A. H., & Jutfelt, F. (2023). Physiological mechanisms of acute upper thermal tolerance in fish. *Physiology*, *38*(3), 141–158. https://doi.org/10.1152/physiol.00027.2022

Farias, I. P., & Fonseca de Almeida-Val, V. M. F. (1992). Malate dehydrogenase (sMDH) in Amazon cichlid fishes: Evolutionary features. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *103*(4), 939–943. https://doi.org/10.1016/0305-0491(92)90219-H

Ferreira, N. C. D. A., De Almeida-Val, V. M. F., & Schwantes, M. L. B. (1991). Lactate dehydrogenase (LDH) in 27 species of Amazon fish: Adaptive and evolutive aspects. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *100*(2), 391–398. https://doi.org/10.1016/0305-0491(91)90392-Q

Ficke, A. D., Myrick, C. A., & Hansen, L. J. (2007). Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries*, *17*(4), 581–613. https://doi.org/10.1007/s11160-007-9059-5

Goward, C. R., & Nicholls, D. J. (1994). Malate dehydrogenase: A model for structure, evolution, and catalysis. *Protein Science*, *3*(10), 1883–1888. https://doi.org/10.1002/pro.5560031027

Guillen, A. C., Borges, M. E., Herrerias, T., Kandalski, P. K., de Arruda Marins, E., et al. (2019). Effect of gradual temperature increase on the carbohydrate energy metabolism responses of the Antarctic fish *Notothenia rossii*. *Marine Environmental Research*, *150*, 104779. https://doi.org/10.1016/j.marenvres.2019.104779

Hochachka, P. W. (1965). Isoenzymes in metabolic adaptation of a poikilotherm: Subunit relationships in lactic dehydrogenases of goldfish. *Archives of Biochemistry and Biophysics*, *111*(1), 96–103. https://doi.org/10.1016/0003-9861(65)90327-9

Katwate, U., Raut, R., Khot, M., Paingankar, M., & Dahanukar, N. (2012). Molecular identification and ecology of a newly discovered population of sun catfish *Horabagrus brachysoma* from northern Western Ghats of India. *ISRN Zoology*, *2012*, 1–9. https://doi.org/10.5402/2012/419320

Kent, J., Koban, M., & Prosser, C. L. (1988). Cold-acclimation-induced protein hypertrophy in channel catfish and green sunfish. *Journal of Comparative Physiology. Part B*, *158*(2), 185–198. https://doi.org/10.1007/BF01075832

Koenekoop, L., & Åqvist, J. (2023). Principles of cold adaptation of fish lactate dehydrogenases revealed by computer simulations of the catalytic reaction. *Molecular Biology Evolution*, *40*(5), msad099. https://doi.org/10.1093/molbev/msad099

Lannig, G., Eckerle, L., Serendero, I., Sartoris, F.-J., Fischer, T., Knust, R., Johansen, T., & Pörtner, H.O. (2003). Temperature adaptation in eurythermal cod (*Gadus morhua*): A comparison of mitochondrial enzyme capacities in boreal and Arctic populations. *Marine Biology*, *142*(3), 589–599. https://doi.org/10.1007/s00227-002-0967-6

Li, Q. Q., Zhang, J., Wang, H. Y., Niu, S. F., Wu, R. X., Tang, B. G., Wang, Q. H., Liang, Z. B., & Liang, Y. S. (2023). Transcriptomic response of the liver tissue in Trachinotusovatus to acute heat stress. *Animals*, *13*(13), 2053. https://doi.org/10.3390/ani13132053

Lin, J. J., & Somero, G. N. (1995a). Thermal adaptation of cytoplasmic malate dehydrogenases of eastern Pacific barracuda (*Sphyraena*spp): The role of differential isoenzyme expression. *Journal of Experimental Biology*, *198*(2), 551–560. https://doi.org/10.1242/jeb.198.2.551

Lin, J. J., & Somero, G. N. (1995b). Temperature-dependent changes in expression of thermostable and thermolabile isozymes of cytosolic malate dehydrogenase in the eurythermal goby fish *Gillichthys mirabilis*. *Physiological Zoology*, *68*(1), 114–128. https://doi.org/10.1086/physzool.68.1.30163921

Luo, L., Zhao, Z., Zhang, R., Guo, K., Wang, S., Xu, W., & Wang, C. (2022). The effects of temperature changes on the isozyme and Hsp70 levels of the Amur sturgeon, *Acipenser schrenckii*, at two acclimation temperatures. *Aquaculture*, *551*, 737743. https://doi.org/10.1016/j.aquaculture.2021.737743

Markert, C. L., & Faulhaber, I. (1965). Lactate dehydrogenase isozyme patterns of fish. *Journal of Experimental Zoology*, *159*(3), 319–332. https://doi.org/10.1002/jez.1401590304

McCaw, B. A., Stevenson, T. J., & Lancaster, L. T. (2020). Epigenetic responses to temperature and climate. *Integrative and Comparative Biology*, *60*(6), 1469–1480. https://doi.org/10.1093/icb/icaa049

McKenzie, D. J., Zhang, Y., Eliason, E. J., Schulte, P. M., Claireaux, G., Blasco, F. R., Nati, J. J. H., & Farrell, A. P. (2021). Intraspecific variation in tolerance of warming in fishes. *Journal of Fish Biology*, *98*(6), 1536–1555. https://doi.org/10.1111/jfb.14620

Monteiro, M. D. C., Schwantes, M. L. B., Schwantes, A. R., & Silva, M. R. D. A. (1998). Thermal stability of soluble malate dehydrogenase isozymes of subtropical fish belonging to the orders Characiformes, Siluriformes and Perciformes. *Genetics and Molecular Biology*, *21*(2), 191–199. https://doi.org/10.1590/S1415-47571998000200004

Morgan, R., Andreassen, A. H., Åsheim, E. R., Finnøen, M. H., Dresler, G., Brembu, T., Loh, A., Miest, J. J., & Jutfelt, F. (2022). Reduced physiological plasticity in a fish adapted to stable temperatures. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(22), e2201919119. https://doi.org/10.1073/pnas.2201919119

Murphy, R. W., Sites, J. W., Buth, D. G., & Haufler, C. H. (1990). Proteins I: isozyme electrophoresis. In D. M. Hillis & C. Moritz (Eds.), *Molecular systematics* (pp. 45–126). Sinauer Associates, Sunderland, MA.

Ozernyuk, N. D., Klyachko, O. S., & Polosukhina, E. S. (1994). Acclimation temperature affects the functional and structural properties of lactate dehydrogenase from fish (*Misgurnusfossilis*) skeletal muscles. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *107*(1), 141–145. https://doi.org/10.1016/0305-0491(94)90236-4

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J., & Britton-Davidian, J. (1988). *Practical isozyme genetics*. Ellis Horwood Ltd., Chichester, England.

Poly, W. J. (1997). Nongenetic variation, genetic-environmental interactions and altered gene expression. I. Temperature, photoperiod, diet, pH and sex-related effects. *Comparative Biochemistry and Physiology. Part A, Physiology*, *117*(1), 11–66. https://doi.org/10.1016/s0300-9629(96)00366-0

Powers, D. A., & Schulte, P. M. (1998). Evolutionary adaptations of gene structure and expression in natural populations in relation to a changing environment: A multidisciplinary approach to address the million-year saga of a small fish. *Journal of Experimental Zoology*, *282*(1–2), 71–94. https://doi.org/10.1002/(SICI)1097-010X(199809/10)282:1/2<71::AID-JEZ11>3.0.CO;2-J

Raghavan, R., Philip, S., Ali, A., Katwate, U., & Dahanukar, N. (2016). Fishery, biology, aquaculture and conservation of the threatened Asian Sun catfish. *Reviews in Fish Biology and Fisheries*, *26*(2), 169–180. https://doi.org/10.1007/s11160-016-9418-1

Rao, M. R. K., Padhi, B. K., & Khuda-Bukhsh, A. R. (1989). Lactate dehydrogenase isozymes in fifty-two species of teleostean fishes: Taxonomic significance of Ldh-C gene expression. *Biochemical Systematics and Ecology*, *17*(1), 69–76. https://doi.org/10.1016/0305-1978(89)90045-8

Schulte, P. M. (2004). Changes in gene expression as biochemical adaptations to environmental change: A tribute to Peter Hochachka. *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology*, *139*(3), 519–529. https://doi.org/10.1016/j.cbpc.2004.06.001

Schwantes, M. L. B., & Schwantes, A. R. (1982a). Adaptative features of ectothermic enzymes—I. Temperature effects on the malate dehydrogenase from a temperate fish *Leiostomusxanthurus*. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *72*(1), 49–58. https://doi.org/10.1016/0305-0491(82)90009-8

Schwantes, M. L. B., & Schwantes, A. R. (1982b). Adaptative features of ectothermic enzymes—II. The effects of acclimation

temperature on the malate dehydrogenase of the spot, *Leiostomusxanthurus*. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *72*(1), 59–64. https://doi.org/10.1016/0305-0491(82)90010-4

Seddon, W. L. (1997). Mechanisms of temperature acclimation in the channel catfish Ictalurus punctatus: Isozymes and quantitative changes. *Comparative Biochemistry and Physiology Part A: Physiology*, *118*(3), 813–820. https://doi.org/10.1016/S0300-9629(97)87356-2

Segal, J. A., & Crawford, D. L. (1994). LDH-B enzyme expression: The mechanisms of altered gene expression in acclimation and evolutionary adaptation. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *267*(4), R1150–R1153. https://doi.org/10.1152/ajpregu.1994.267.4.R1150

Sejian, V., Bhatta, R., Gaughan, J. B., Dunshea, F. R., &Lacetera, N. (2018). Review: Adaptation of animals to heat stress. *Animal*, *12*(s2), s431–s444. https://doi.org/10.1017/S1751731118001945

Shaklee, J. B., Allendorf, F. W., Morizot, D. C., & Whitt, G. S. (1990). Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society*, *119*(1), 2–15. https://doi.org/10.1577/1548-8659(1990)119<0002:GNFPLI>2.3.CO;2

Smirnova, Y. A., Zinov'eva, R. D., & Ozernyuk, N. D. (2002). Effect of thermal acclimation on the expression of gene coding for lactate dehydrogenase A 4. In Loach Skeletal Muscle. *Biology bulletin of the Russian Academy of Sciences*, *29*, 207–211.

Somero, G. N. (1973). Thermal modulation of pyruvate metabolism in the fish *Gillichthys mirabilis*: The role of lactate dehydrogenases. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *44*(1), 205-206. https://doi.org/10.1016/0305-0491(73)90357-x

Somero, G. N. (2004). Adaptation of enzymes to temperature: Searching for basic "strategies".*Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology*, *139*(3), 321–333. https://doi.org/10.1016/j.cbpc.2004.05.003

Somero, G. N. (1975). The role of isozymes to adaptation varying temperature. In C. L. Markert (Ed.), *Isozymes II. Physiological function* (pp. 221–234). Academic Press, New York.

Sureshkumar, S., Ranjeet, K., & Radhakrishnan, K. V. (2013). Live handling and domestication of selected indigenous ornamental fishes of India. *International Journal of Fisheries and Aquatic Studies*, *1*(5), 08–11.

Tattersall, G. J., Sinclair, B. J., Withers, P. C., Fields, P. A., Seebacher, F., Cooper, C. E., & Maloney, S. K. (2012). Coping with thermal challenges: Physiological adaptations to environmental temperatures. *Comprehensive Physiology*, *2*(3), 2151–2202. https://doi.org/10.1002/cphy.c110055

Triveni, A., & Rao, P. R. (1986). Tissue distribution and characterization of LDH isozymes in two fishes of the order Cypriniformes. *Proceedings of Indian Academy of Science: Animal Sciences*, *95*(2), 255–262. https://doi.org/10.1007/BF03179584

Volkoff, H., & Rønnestad, I. (2020). Effects of temperature on feeding and digestive processes in fish. *Temperature*, *7*(4), 307–320. https://doi.org/10.1080/23328940.2020.1765950

Vornanen, M. (1994). Seasonal adaptation of crucian carp (*Carassius carassius* L.) heart: Glycogen stores and lactate dehydrogenase activity. *Canadian Journal of Zoology*, *72*(3), 433–442. https://doi.org/10.1139/z94-061

Walker, J. M. (2002). Nondenaturing polyacrylamide gel electrophoresis of proteins. In J. M. Walker (Ed.), *The Protein Protocols Handbook* (pp. 57–60). Humana Press, Totowa, N.J.

Wilson, F. R., Champion, M. J., Whitt, G. S., & Prosser, C. L. (1975). Isozyme patterns of in tissues of temperature-acclimated fishes. In C. L. Market (Ed.), *Isozymes II, Physiological function* (pp. 193–206). Academy Press, New York.

Zakhartsev, M., Johansen, T., Pörtner, H. O., & Blust, R. (2004). Effects of temperature acclimation on lactate dehydrogenase of cod (*Gadus morhua*): Genetic, kinetic and thermodynamic aspects. *Journal of Experimental Biology*, *207*(1), 95–112. https://doi.org/10.1242/jeb.00708