

Sage Sularz

Comparative Physiology

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Effect of Species and Temperature on MDH Enzyme Activity

Tissue Collection and Homogenization

Freshwater mussels (*Mytilus* sp.) and crayfish (*Procambarus* sp.) were collected for this experiment. Dissections were performed on specific muscle tissues: the adductor muscle in mussels and the tail muscle in crayfish. Dissected tissues were immediately placed on ice to preserve enzyme activity. Each tissue sample was weighed on a tared analytical balance. To prepare homogenates, tissues were minced and homogenized in a 50 mM potassium phosphate buffer (pH 6.8) at a 1:4 tissue-to-buffer ratio, ensuring a consistent 5X dilution. After homogenization, samples were centrifuged at 14,000 x g for 5 minutes at 4°C to remove insoluble material. The resulting supernatant was collected for enzyme assays.

Measuring Malate Dehydrogenase (MDH) Activity

MDH activity was measured using a spectrophotometric assay that tracked the decrease in NADH absorbance at 340 nm. This absorbance reduction indicates MDH activity as NADH is oxidized to NAD⁺. The assay cocktail contained 0.20 M imidazole/Cl buffer (pH 7.0), 0.15 mM NADH, and 0.2 mM oxaloacetate. All reagents were freshly prepared and stored on ice to minimize degradation. Each assay was conducted in triplicate to ensure data accuracy. For each trial, 2.0 mL of reaction cocktail was thermally equilibrated at one of the target temperatures (15°C, 25°C, or 35°C) in a water bath. After reaching equilibrium, 25 µL of the tissue homogenate was added to initiate the reaction. The change in absorbance at 340 nm was monitored for 3 minutes, and enzyme activity was calculated based on the linear portion of the reaction curve, accounting for product inhibition of MDH.

Calculation of MDH Activity

MDH activity was calculated as International Units (I.U.) per gram of fresh tissue weight (gfw) using the following formula:

$$I.U./gfw = \frac{\Delta Absorbance/min \times 0.322}{gfw}$$

where I.U. is the international units of enzyme activity, 0.322 is a factor accounting for the volume of the reaction mixture (2.00 mL) and gfw is the gram fresh weight of tissue used in the assay. The average MDH activity for each species was calculated for each temperature.

Temperature Dependence of MDH Activity and Q10 Calculation

To determine the effect of temperature on MDH activity, assays were conducted at three temperatures: 15°C, 25°C, and 35°C. Q10 values were calculated to quantify the temperature sensitivity of the enzyme's activity over two temperature ranges: 15°C to 25°C and 25°C to 35°C. The Q10 value was calculated using the formula:

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\frac{10}{T_2 - T_1}}$$

where R2 and R1 are the average MDH activities at the higher and lower temperatures, respectively, and T2 and T1 are the corresponding temperatures in degrees Celsius.

Statistical Analysis

A two-way ANOVA was performed to assess the effects of temperature and species on MDH activity. This analysis tested for significant differences in enzyme activity between species (mussel and crayfish) and across temperatures (15°C, 25°C, and 35°C). Post-hoc tests were conducted to determine significant differences between specific temperature and species pairs. All statistical analyses were performed using R statistical software, and p-values less than 0.05 were considered statistically significant.

Supplemental Information

Citations

Wickham H, François R, Henry L, Müller K (2023). dplyr: A Grammar of Data Manipulation. R package version 1.1.3, <https://CRAN.R-project.org/package=dplyr>.

Wickham H, Girlich M (2023). tidyr: Tidy Messy Data. R package version 1.3.0, <https://CRAN.R-project.org/package=tidyr>.

Figures

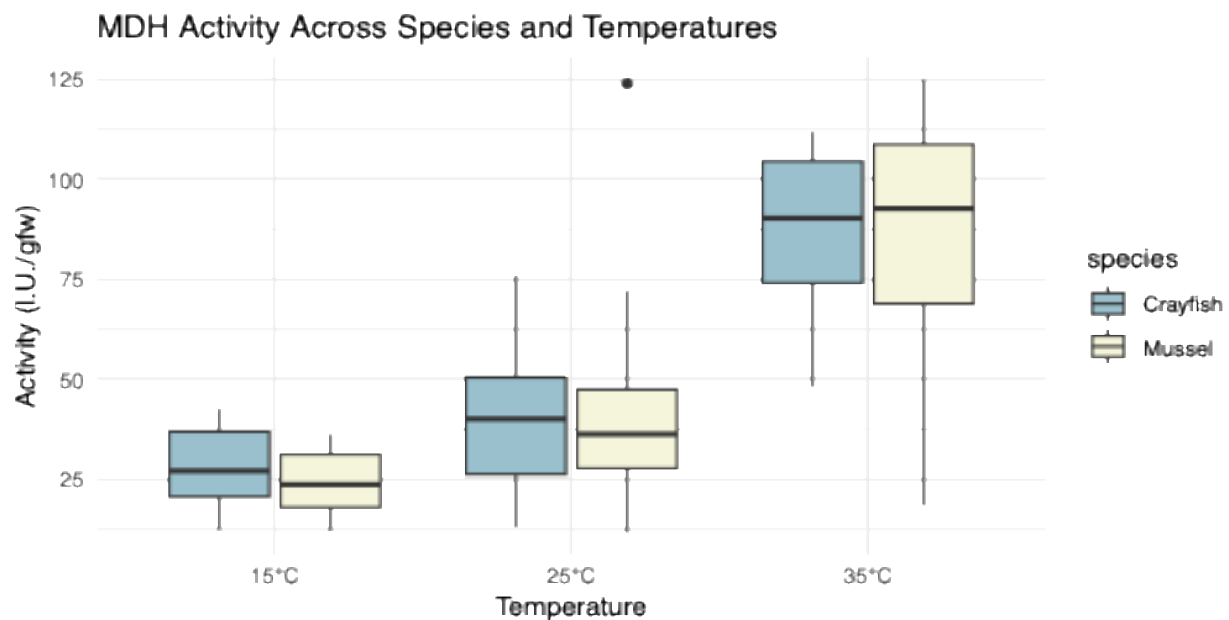


Figure 1: Boxplot shows MDH activity (I.U./gfw) in crayfish and mussel muscle tissue measured at three temperatures: 15°C, 25°C, and 35°C. Enzyme activity increased with temperature for both species, with crayfish generally displaying higher MDH activity than mussels at 35°C. *Q10* values for crayfish were 1.43 (15–25°C) and 2.18 (25–35°C), while mussels showed *Q10* values of 1.81 (15–25°C) and 2.01 (25–35°C), indicating greater temperature sensitivity in the higher range for both species.

A two-way ANOVA revealed a significant effect of temperature on MDH activity ($F(2, 60) = 45.264$, $p < 0.001$). However, there was no significant effect of species ($F(1, 60) = 0.000$, $p = 0.997$) nor an interaction between temperature and species ($F(2, 60) = 0.156$, $p = 0.856$), suggesting that temperature increases enzyme activity similarly in both species.