

Biomonitoring of water lice *Asellus aquaticus* from cave and surface freshwater environments

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Introduction

The water louse, *Asellus aquaticus* (L.) is an ubiquitous freshwater isopod crustacean, included in biological processes in the benthic community (Hasu *et al.*, 2007). Many subspecies have been described from Slovenia, inhabiting a wide array of surface and subterranean environments (Sket, 1994).

The goal of this research is to investigate selected biochemical parameters (biomarkers) in water lice from different environments and to establish the differences in relation to their environment and season. The studied biomarkers are energy reserves (lipids and carbohydrates) and enzyme activities of acetylcholinesterase (AChE) and glutathione S-transferase (GST). AChE is involved in numerous neuronal and non-neuronal functions while GST is a xenobiotic biotransformation enzyme.

Only the results from the first sampling (March 2015) are presented.

Methods & Materials

Samples of three *Asellus aquaticus* subspecies were collected along the sinking river Pivka: from its surface section at the entrance of Postojnska jama Cave (*A. a. aquaticus*), its underground section in Planinska jama Cave (*A. a. cavernicolus*) and its resurgence called the Unica River on Planinsko Polje (*A. a. carniolicus*). Another sample of the type subspecies was collected from a swampy forest in Ljubljana.

Lipids and carbohydrates were quantified according to Bligh and Dyer (1959) and Albalasmeh *et al.* (2013), respectively. AChE and GST enzyme activities were determined according to Ellman *et al.* (1961) and Habig *et al.* (1974), respectively. Fifteen individuals were used for each analysis. Gender has not been determined yet, but no ovigerous females were collected.

Statistical analysis was performed in R 3.1.2. Significant differences were tested with a Kruskal-Wallis rank sum test and the post-hoc analyses were conducted with Dunn's Multiple Comparison Test using rank sums. The Tukey box-and-whiskers box plots were generated using the ggplot2 package. Whiskers represent the lowest and highest data still within 1,5 IQR of the lower and upper quartile, respectively.

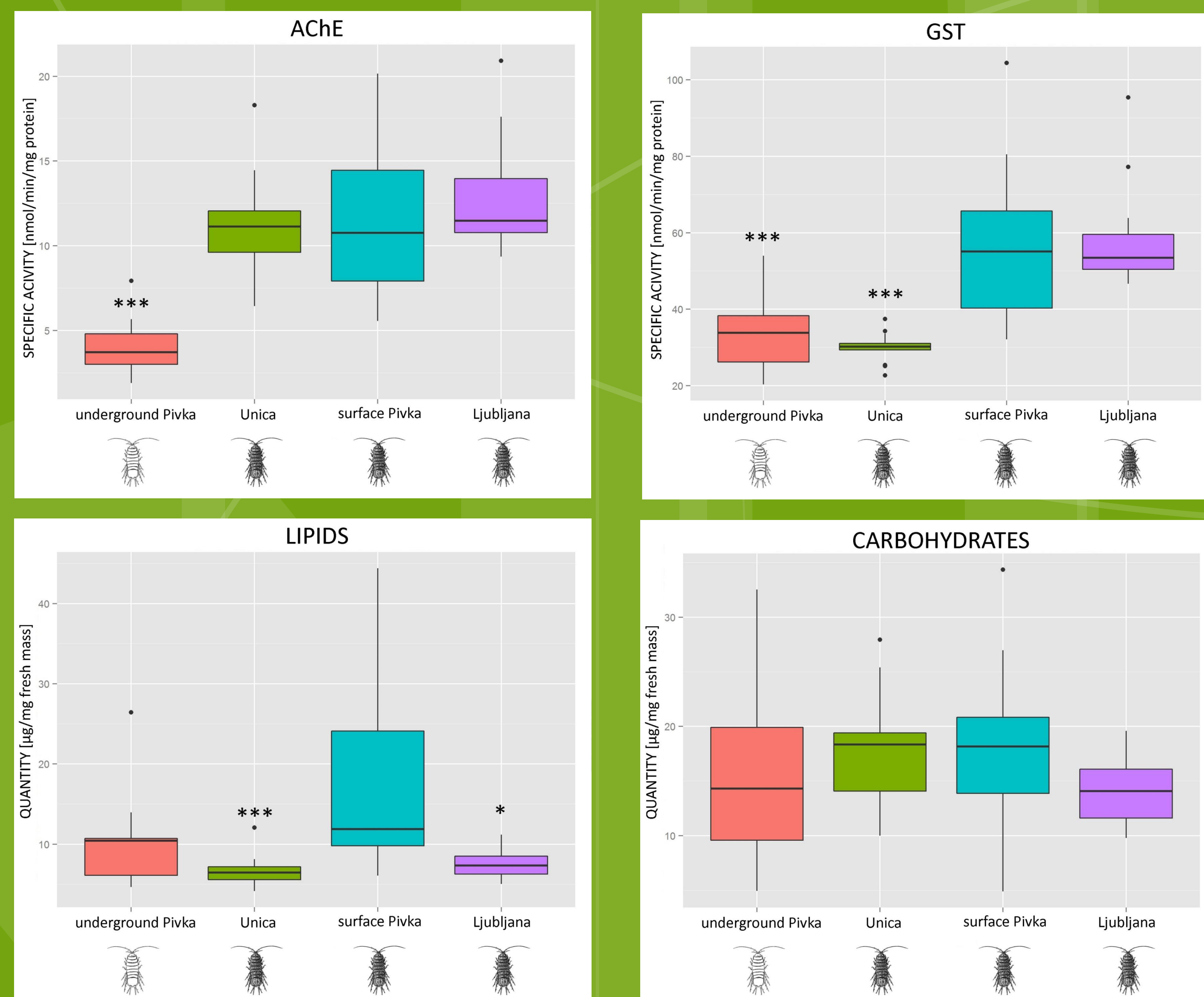
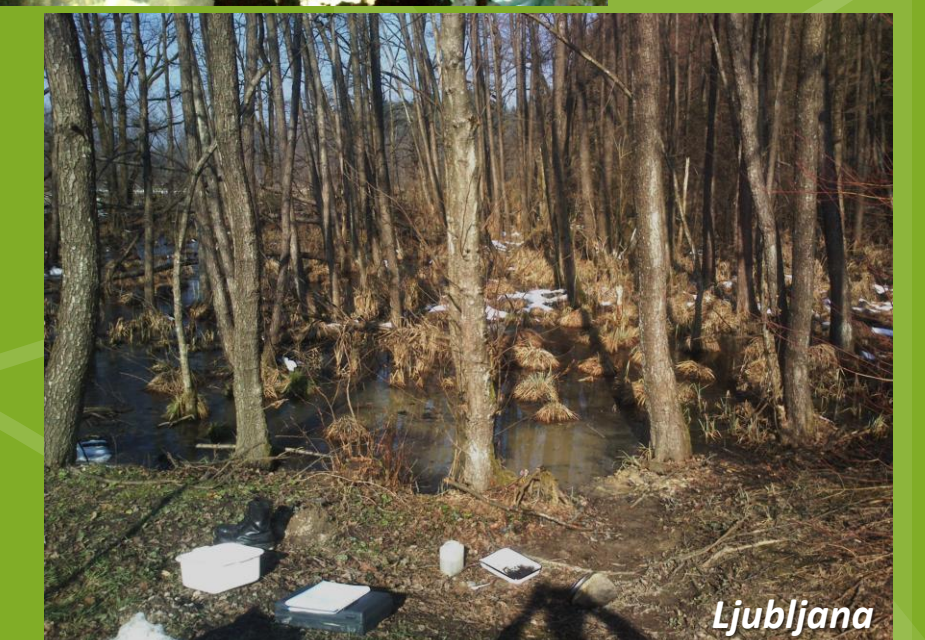
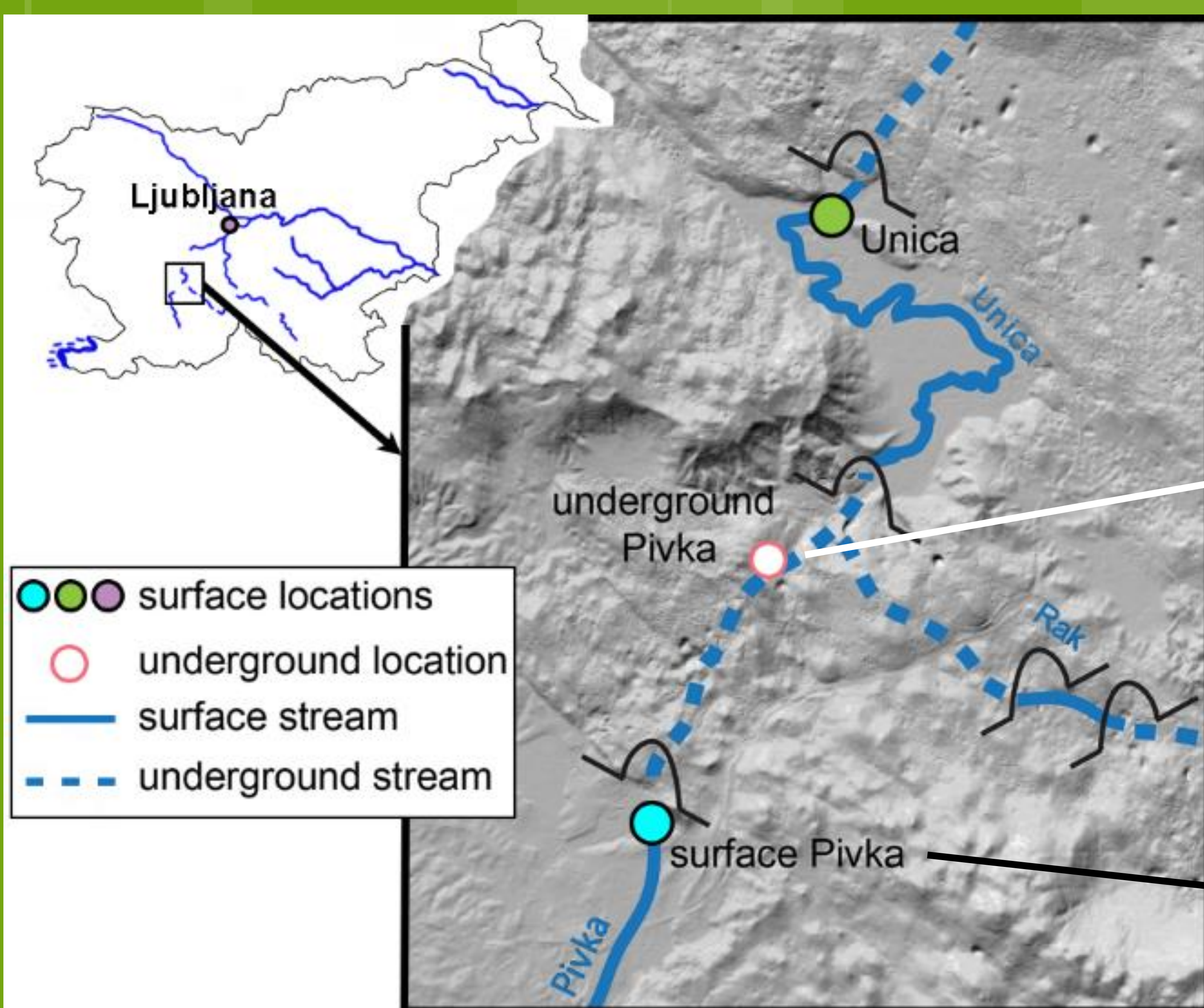


Fig. 1: Acetylcholinesterase (AChE) and glutathione S-transferase (GST) activities and lipid and carbohydrate quantities in cave and surface water lice, *Asellus aquaticus* from Slovenia. Asterisks denote statistically significant differences: $p < 0,05$ (*), $p < 0,001$ (***). N = 15.

Results & Discussion

Significant differences in AChE and GST activities as well as lipid quantities were found among water lice from different locations whereas the differences in their carbohydrate quantities were not significant (Fig. 1).

The significantly lower AChE activity in the water louse from the subterranean Pivka will require further analysis, but it may be due to its lower locomotory activity.

The increased GST activity denotes higher metabolic activity of water lice from the surface Pivka and Ljubljana, potentially indicating organically enriched water.

The observed differences in lipid energy reserves can be connected either to habitat conditions or gender. The latter aspect will be taken into consideration in further experiments.

Two additional sample sets (collected in June/July and October), as well as specimen gender identification may shed more light on the observed differences.

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References

- Albalasmeh, A. A., Berhe, A. A., Ghezzehei, T. A., 2013. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydrate Polymers* 97, 253-261.
- Bligh, E.G., Dyer, W.J., 1959. A Rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37, 911-917.
- Ellman, L.G., Courtney, K.D., Andres Jr., V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* 7, 88-95.
- Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases, the first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry* 249, 7130-7139.
- Hasu, T., Holmes, J. C., Tellervo Valtonen E., 2007. Isopod (*Asellus aquaticus*) size and acanthocephalan (*Acanthocephalus lucii*) infections. *Journal of Parasitology* 93, 450-457.
- Sket, B. 1994. Distribution patterns of some subterranean Crustacean in the territory of the former Yugoslavia. *Hydrobiologia* 287, 65-75.