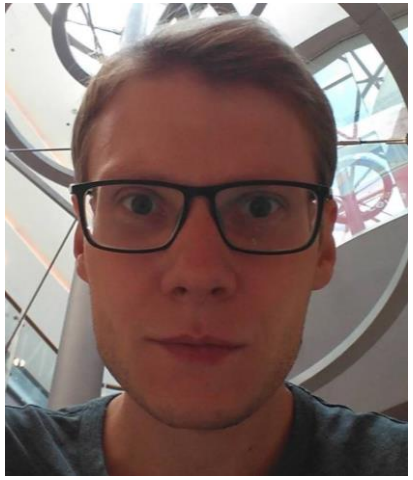
NICOLAUS COPERNICUS
UNIVERSITY
IN TORUŃ

Hemolytic activity of venoms of the water shrew *Neomys fodiens* and the common shrew *Sorex araneus*

Krzysztof Kowalski

Collaborators

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ
Faculty of Biological and Veterinary Sciences



Krzysztof Kowalski, Ph.D.

Nicolaus Copernicus University in Toruń

Leszek Rychlik, Professor

Paweł Marciniak, Ph.D.

Adam Mickiewicz University in Poznań

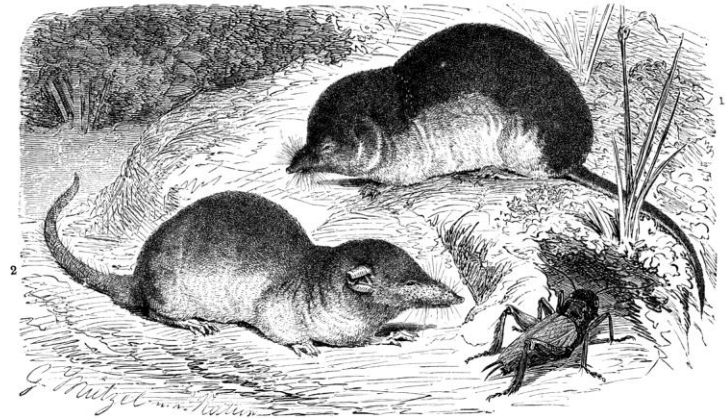
Funding

The research was supported by grant no. 2015/17/N/NZ8/01567 from the National Science Centre (Poland)

NATIONAL SCIENCE CENTRE POLAND

Venomous mammals

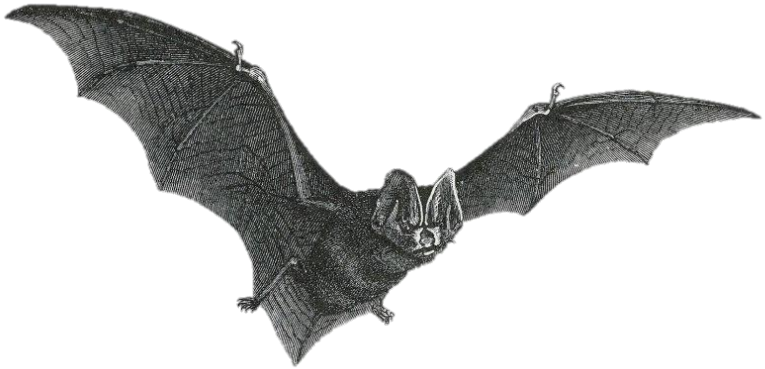
NICOLAUS COPERNICUS
UNIVERSITY
IN TORUŃ
Faculty of Biological
and Veterinary Sciences



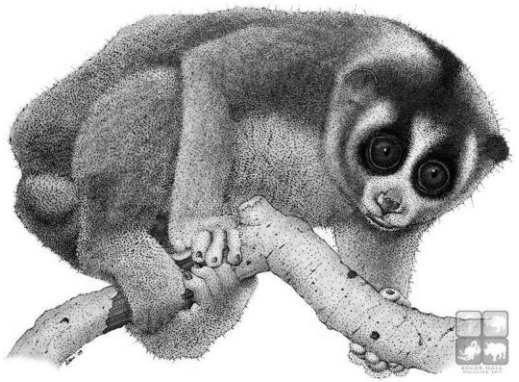
shrews (*Neomys*, *Blarina*)

solenodons

platypus



vampire bats



lorises

Dufton 1992; Ligabue-Braun et al. 2012; Kowalski & Rychlik 2014, 2018; Rode-Margono & Nekaris 2015

Shrew venoms

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ
Faculty of Biological and Veterinary Sciences

blarina toxin, soricidin

kallikrein-1 serine protease

phospholipase A_2

antileukoproteinase

hyaluronidase

↓

irregular respiration

paralysis and convulsions

death

phospholipase A_2

lysozyme C

hyaluronidase

↓

irregular respiration

paralysis and convulsions

death

Kita et al. 2004, 2005; Bowen et al. 2013; Hanf & Chavez 2020

Pucek 1959, 1969
Kowalski et al. 2017

Research objectives

NICOLAUS COPERNICUS
UNIVERSITY
IN TORUŃ
Faculty of Biological
and Veterinary Sciences

- to determine hemolytic activity of saliva of the water shrew *Neomys fodiens* and the common shrew *Sorex araneus*

- to identify toxic components of saliva of *N. fodiens* and *S. araneus*

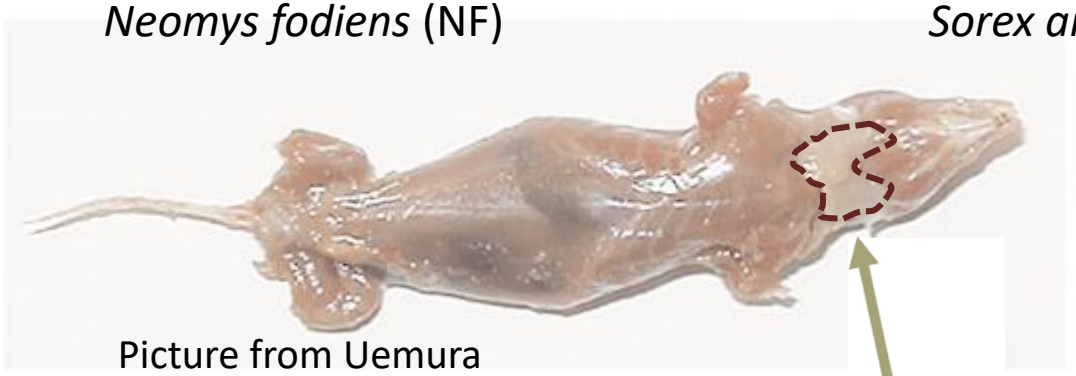
Venom sampling

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ
Faculty of Biological and Veterinary Sciences

extraction of the salivary glands*

Neomys fodiens (NF)

Sorex araneus (SA)



Picture from Uemura et al. 2009

the submandibular glands

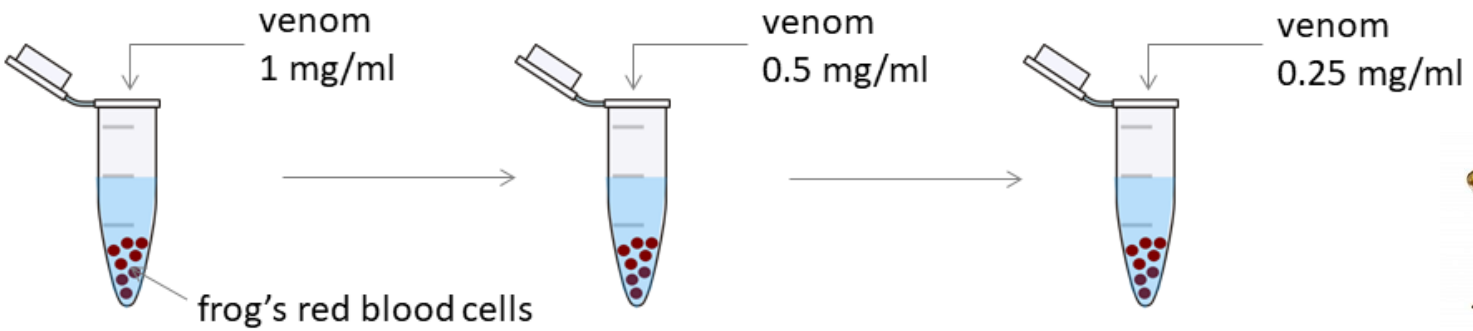
*n=12 for both shrew species

Methods

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ
Faculty of Biological and Veterinary Sciences

Hemolytic assay

- treatment of red blood cells with venom/saliva of NF and SA at concentrations of 1.0, 0.5 and 0.25 mg/ml



frog (*Pelophylax* sp.)

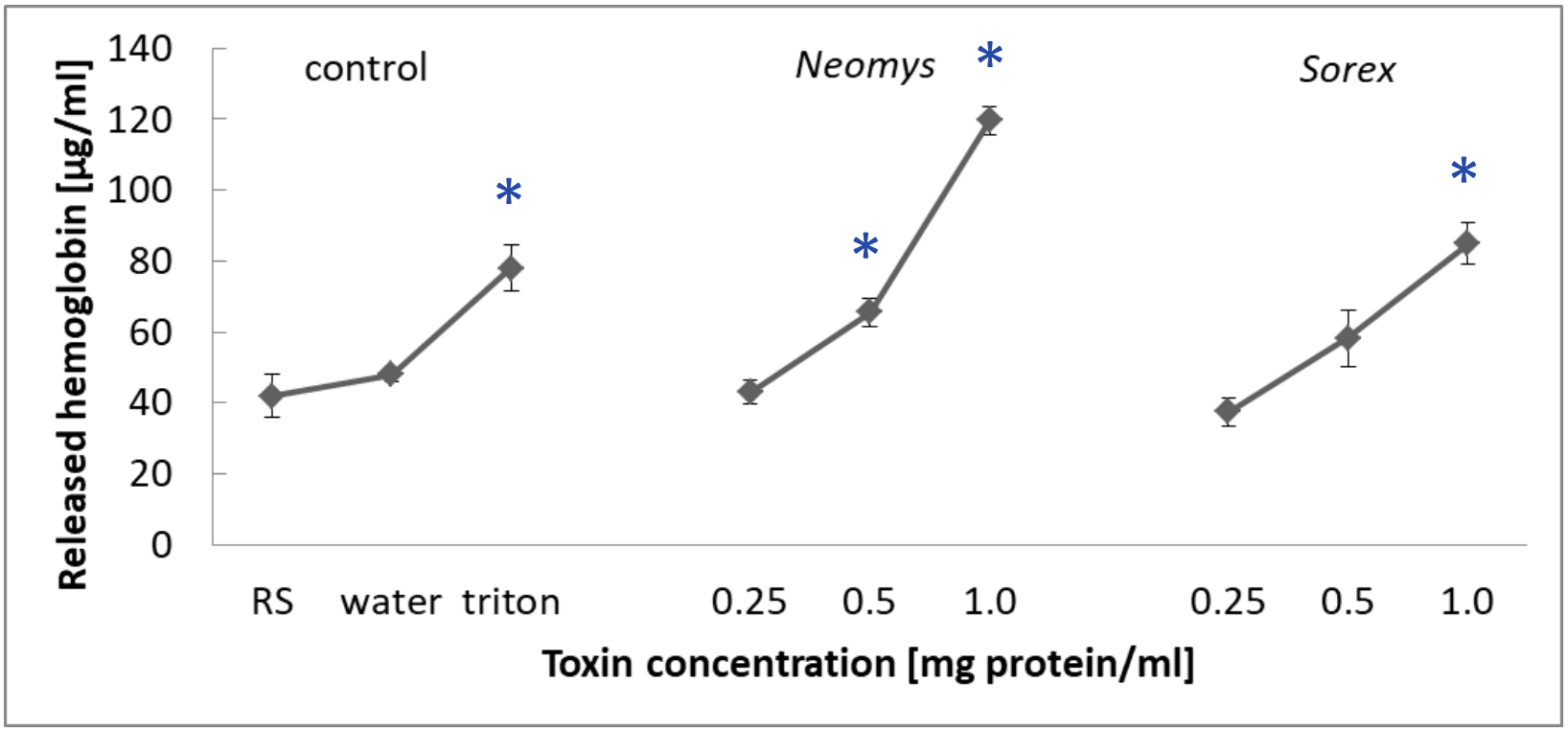
- control tests
  - distilled water and Ringer's solution (RS) – negative controls
  - Triton® X-100 as a positive control

Protein identification

- chromatographic separation and proteomic analysis

Hemolytic activity of shrew venom

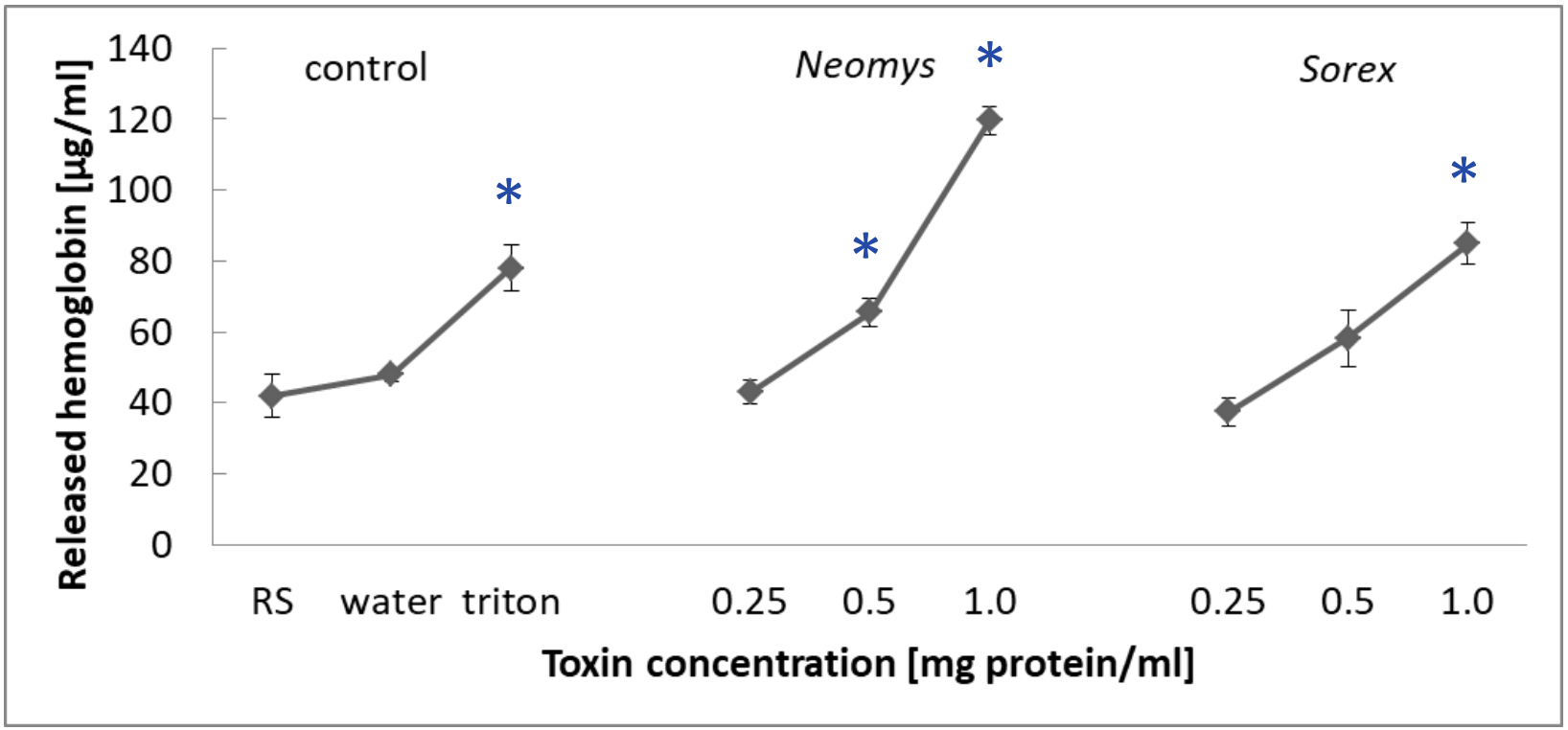
NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ
Faculty of Biological and Veterinary Sciences



- significant concentration-dependent effects of salivary extracts of both shrews on hemolysis in erythrocytes
- hemolytic effects of NF venom were stronger than those produced by SA (Mann-Whitney U-test: $U = 141$, $p < 0.0001$)

Hemolytic activity of shrew venom

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ
Faculty of Biological and Veterinary Sciences

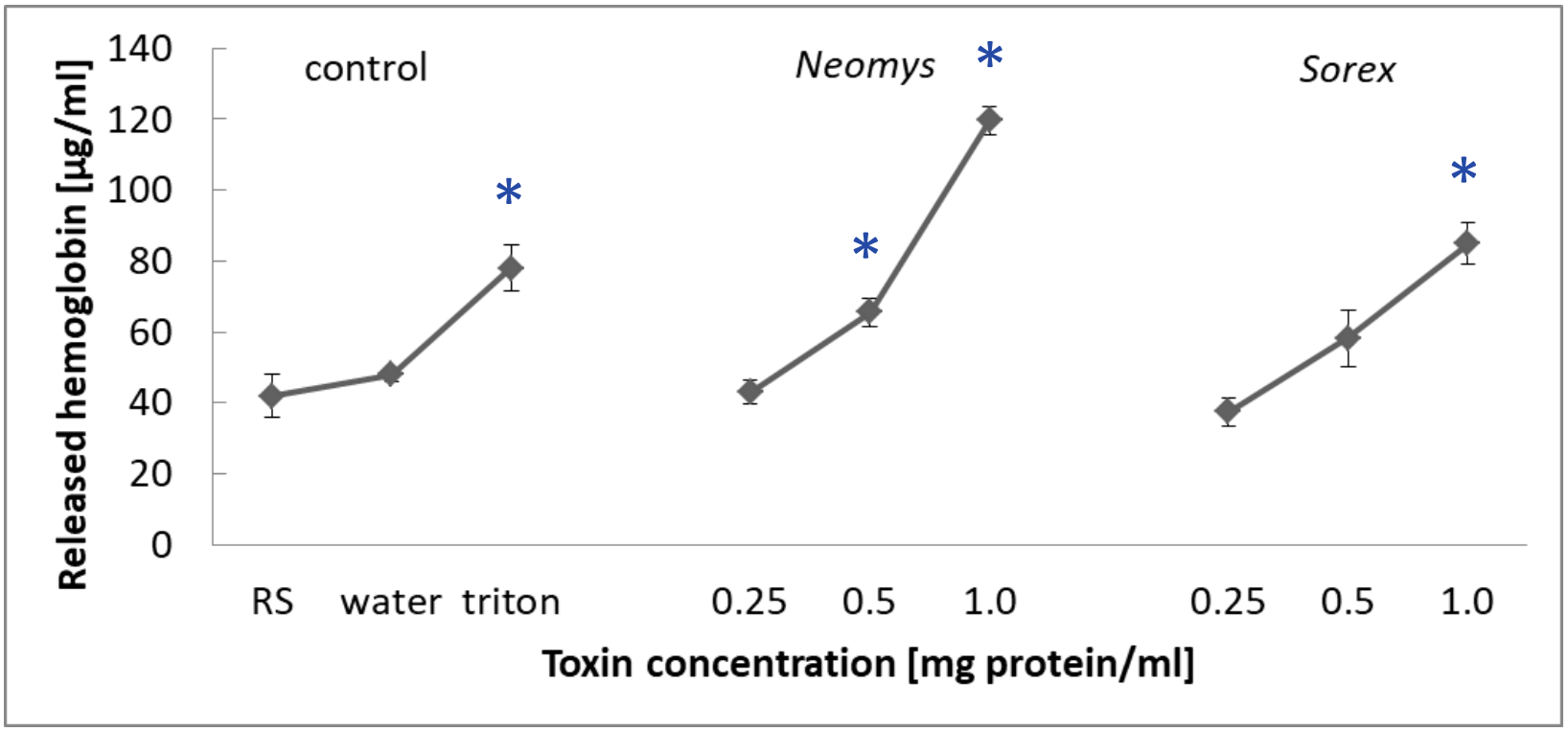


- venom of NF produced hemolysis at concentrations of **0.5** and **1.0 mg/ml**

venom (0.5 mg/ml) vs
- RS (Student's t-test: t = -3.2, df = 28, p = 0.006)
- water (U = 45.5, p < 0.01)
- triton (U = 151, p = 0.17)

Hemolytic activity of shrew venom

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ
Faculty of Biological and Veterinary Sciences

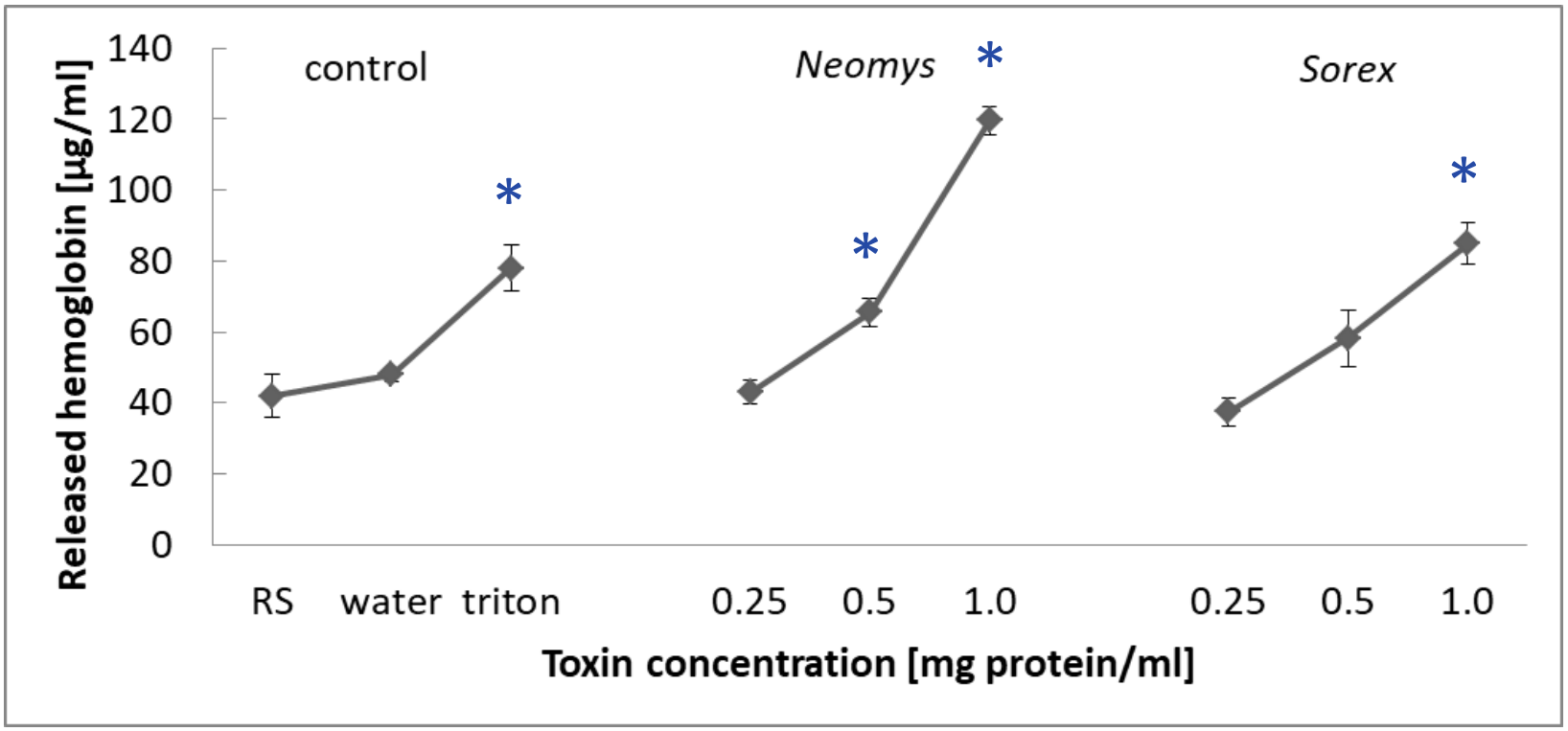


- venom of NF produced hemolysis at concentrations of **0.5** and **1.0 mg/ml**

venom (1.0 mg/ml) vs
RS ($t = -10.7$, $df = 28$, $p < 0.0001$)
water ($U = 0$, $p < 0.0001$)
triton ($U = 18$, $p < 0.0001$)

Hemolytic activity of shrew venom

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ
Faculty of Biological and Veterinary Sciences



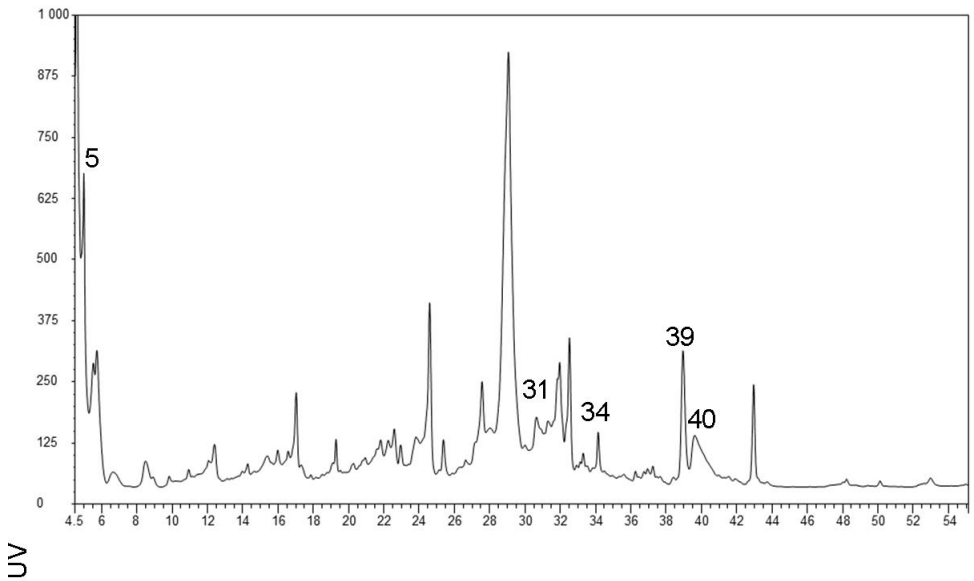
- saliva of SA produced hemolysis at concentration of **1.0 mg/ml**

venom (1.0 mg/ml) vs
- RS (U = 2, p < 0.001)
- water (U = 0, p < 0.001)
- triton (U = 27, p = 0.33)

Toxin identification

NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ
Faculty of Biological and Veterinary Sciences

Neomys fodiens



phospholipase A_2

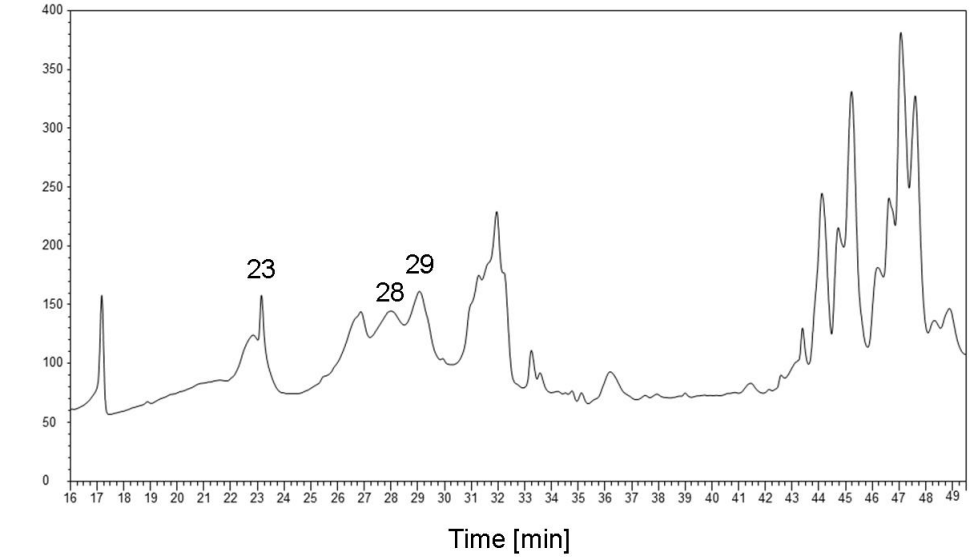
lysozyme C

proenkephalin

disintegrin and metalloproteinase domain-containing protein

hyaluronidase

Sorex araneus



lysozyme C

proenkephalin

kallikrein 1-related peptidase

disintegrin and metalloproteinase domain-containing protein

beta-defensin

Conclusions

NICOLAUS COPERNICUS
UNIVERSITY
IN TORUŃ
Faculty of Biological
and Veterinary Sciences

- shrew venoms, in addition to potent paralytic properties, possess also hemolytic activity that may allow them to hunt larger prey as frogs

- due to the toxic activity of its saliva the common shrew may be considered venomous mammal

References

Bowen et al. *In vivo* detection of human TRPV6-rich tumors with anti-cancer peptides derived from soricidin. *PLOS ONE* **2013**, *8*, e58866.

Dufton, M.J. Venomous mammals. *Pharmacol. Ther.* **1992**, *53*, 199-215.

Hanf, Z.R.; Chavez, A.S. A comprehensive multi-omic approach reveals a relatively simple venom in a diet generalist, the northern short-tailed shrew, *Blarina brevicauda*. *Genome Biol. Evol.* **2020**, *evaa115*.

Kita et al. Blarina toxin, a mammalian lethal venom from the short-tailed shrew *Blarina brevicauda*: Isolation and characterization. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 7542-7547.

Kita et al. Purification and characterization of blarinasin, a new tissue kallikrein-like protease from the short-tailed shrew *Blarina brevicauda*: comparative studies with blarina toxin. *Biol. Chem.* **2005**, *386*, 177-182.

Kowalski, K.; Marciniak, P.; Rosiński, G.; Rychlik, L. Evaluation of the physiological activity of venom from the Eurasian water shrew *Neomys fodiens*. *Front. Zool.* **2017**, *14*, 46.

Kowalski, K.; Rychlik, L. Jadowite ssaki [Venomous mammals]. *Kosmos* **2014**, *63*, 643-655.

Kowalski, K.; Rychlik, L. The role of venom in the hunting and hoarding of prey differing in body size by the Eurasian water shrew, *Neomys fodiens*. *J. Mammal.* **2018**, *99*, 351–362.

Ligabue-Braun, R.; Verli, H.; Carlini, C.R. Venomous mammals: A review. *Toxicon* **2012**, *59*, 680-695.

Pucek, M. The effect of the venom of the European water shrew (*Neomys fodiens fodiens* Pennant) on certain experimental animals. *Acta Theriol.* **1959**, *3*, 93-108.

Pucek, M. *Neomys anomalus* Cabrera, 1907 – a venomous mammal. *Bull. De L'Académie Polonaise Des Sci.* **1969**, *17*, 569-573.

Rode-Margono, J.E.; Nekaris, K.A.I. Cabinet of curiosities: Venom systems and their ecological function in mammals, with a focus on primates. *Toxins* **2015**, *7*, 2639-2658.

Uemura, D.; Kita, M.; Arimoto, H.; Kitamura, M. Recent aspects of chemical ecology: Natural toxins, coral communities, and symbiotic relationships. *Pure Appl. Chem.* **2009**, *81*, 1093-1111.

Thank you for your attention…☺

k.kowalski@umk.pl