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Towards the SDG Challenges

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lecular weight pattern of proteins.

CONCLUSIONS:

The results in term of digestosomatic index, specific activities of digestive enzymes, and chyme biochemical characteristics physiologically responded with post-prandial changes in bigfin reef squids. The food transited rapidly and was maximally digested by enzymes within 0.5 to 4 h after feeding. Findings from the current study provide basic information on digestive physiology of bigfin reef squids that can be applied when designing the specimen harvesting time for biological research.

T1-P-22 Acute toxicity assessment of defense secretions of *Megaphyllum bosniense* (Verhoeff, 1897) and *M. Unilineatum* (C. L. Koch, 1838) (Diplopoda, Julida) on *Artemia Salina*

Jelena Milovanović[76], Jelena Đorđević[77/78], Aleksandra Stevanović[78], Anastasija Malešević[78], Bojan Ilić[76], Branka Vuković-Gačić[78], Slobodan Makarov[76]

KEYWORDS: millipedes; Julidae; allomones; biological activities; ARC test

INTRODUCTION:

Different orders within the class Diplopoda possess a variety of chemical compounds in their defense secretions: quinones, phenolics, alcohols, aldehydes, ketones, esters, alkaloids, cyanogenic compounds. Defensive secretions of species from the order Julida are regarded as the most complex within Diplopoda, and they are blends of several classes of chemical compounds: quinones, esters and ketones. Numerous biological activities of these secretions have been reported: antimicrobial, antioxidative, antineurodegenerative, cytotoxic and embryotoxic on zebrafish. Besides zebrafish embryos, *Artemia salina* is one of the common model organisms in toxicity assessment which has not been used for screening of toxicity of millipedes' defensive secretions.

OBJECTIVES:

The main goal of this study was to examine the toxic effects of defensive secretions of two species from the order Julida [*Megaphyllum bosniense* (MBO) and *M. unilineatum* (MUN)] using *Artemia salina* (ARC test).

METHOD / DESIGN:

Adult individuals of *M. bosniense* were collected during April and May of 2021 on Mt. Avala, near Belgrade, while adults of *M. unilineatum* were collected during the same period in the Krnjača, suburb of Belgrade. After the capture, millipedes were kept in plastic boxes containing ground cover from the collecting site. The boxes were regularly sprayed with water to maintain high humidity. Due to the fact that the sample was female-biased, defensive secretions of female specimens were used for further analyses. Excretion of defensive secretions was elicited from glands of mentioned species via mechanical stress in closed glass vials. Secretions collected from both species were dissolved in 10 ml of hexane, concentrated under reduced pressure in a rotary evaporator (Rotavapor R-210, Buchi) at 40°C to a dry residue, and redissolved in 50% dimethyl

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sulphoxide (DMSO). The stock concentration of extracts used in ARC test was 20 mg/mL. Before treatments, eggs of *A. salina* were incubated for 72h with constant lighting and aeration. For the purposes of the experiment, stage II and III larvae were used (separated by phototaxis in 300 ml of seawater). In a plate with 24 wells, 900 µl of seawater with larvae (10-15 per well) was placed and then 100 µl of tested extracts (range of concentrations 0,1 mg/mL - 0,003125 mg/mL) was added. Potassium dichromate ($K_2Cr_2O_7$) was used as a positive control and DMSO was used as solvent control. The total number of individuals per well was counted after 24h and 48h, as well as the number of living and dead individuals. These data were used for estimation of survival rate and determination of LC_{50} value. The experiment was done in triplicate.

RESULTS:

Our results show that secretions from both species exhibit a toxic effect on the survival of the chosen model organism, with the MBO extract showing weaker activity in comparison with MUN extract. The LC_{50} value after 24h was about the same for both species (LC_{50}=73,23 µg/mL for MBO and LC_{50}=68,56 µg/mL for MUN). The LC_{50} value for MBO after 48h was 47,18 µg/mL, while LC_{50} value in the same period for MUN was 29,12 µg/mL. Positive control (LC_{50}= 13,5 µg/mL) showed three times stronger effects in relation to MBO and twice as strong when compared to MUN extract. It has also been shown that the number of surviving individuals decreases with increasing concentration of tested extracts and the increasing incubation time.

CONCLUSIONS:

The defense secretions of both tested millipede species show toxic effects in the ARC test. It is shown that MBO extract has a weaker toxic effect than the MUN extract. This result can be linked with the fact that esters of long-chain fatty acids are dominant compounds in MBO, while MUN is almost exclusively benzoquinone-based. Esters detected in MBO are generally regarded as low-toxic compounds, but with the potential to interact with compounds from other chemical classes. However, as MBO achieved toxic effects and many esters that are detected in MBO are new natural products and their biological potential is unknown, further extensive studies are needed to determine their toxicological potential.

T1-P-23 Preliminary modification of the Eshippo Crayfish model

Simona Đuretanović[79], Tijana Veličković[79], Aleksandra Milošković[80], Nataša Kojadinović[79], Marija Jakovljević[79], Ivana Maguire[81], Vladica Simić[79]

KEYWORDS: *Astacus astacus*; Balkan Peninsula; Species conservation; Conservation models; Multidisciplinary approach

INTRODUCTION:

The extinction of species and the decline of biodiversity are the most severe global consequences of environmental threats. The decline of biodiversity is far greater in freshwater ecosystems than in the most threatened terrestrial ecosystems, and the most vulnerable are invertebrates, such as freshwater crayfish. Even one-third of freshwater crayfish worldwide are at risk of extinction. Natural subpopulations of the noble crayfish have been declining by 50-70%, and it is classified as a "vulnerable species" in the IUCN Red List of Threatened Species, with a decreasing trend of populations and subpopulations and decreasing distribution areas.

We used our published morphometric, phylogenetic, and population genetic data of the noble crayfish populations from aquatic ecosystems of Serbia, Slovenia, and Albania in order to upgrade the existing ESHIPPO crayfish model, and in this way

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