

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<sup>79</sup>, Tijana Veličković<sup>79</sup>, Aleksandra Milošković<sup>80</sup>, Nataša Kojadinović<sup>79</sup>, Marija Jakovljević<sup>79</sup>, Ivana Maguire<sup>81</sup>, Vladica Simić<sup>79</sup>

**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

<sup>79</sup> Faculty of Science, University of Kragujevac, Serbia. Corresponding author: simona.djuretanovic@pmf.kg.ac.rs

<sup>80</sup> Institute for Information Technologies, University of Kragujevac, Serbia

<sup>81</sup> Faculty of Science, Department of Biology, University of Zagreb, Croatia

to contribute to conservation plans and management strategies for protection of this threatened species.

### OBJECTIVES:

The main objectives of this study were:

1. to modify the ESHIPPO crayfish model,
2. to assess the risk of extinction, and
3. to determine the priority of protection in the study area.

### METHOD/DESIGN:

In this study, we upgraded ESHIPPO crayfish model by adding population genetic component of the studied populations. This model is designed to assess the risk of extinction and define the priorities of species conservation in aquatic ecosystems at the local and national levels since frequent differences exist in the assessment of the risk of extinction at the local and global levels.

### RESULTS:

According to the obtained results, the populations from Lake Prespa (68 points), and the Gazivode reservoir (62 points) are defined as populations with a high level of extinction risk at the national level, i.e., the degree of protection priority 1. On the other hand, a moderate risk of extinction, i.e., the degree of protection priority 2, was determined for the populations from the reservoirs Grlišće (58 points), Korenica (56 points), and Bloke (56 points), and for the Kočevska River (52 points).

### CONCLUSIONS:

We determined six populations as the priority of protection. This kind of model can help identify and preserve the diversity of the species and the integrity of local populations.

## T1-P-24 Estimation of the wastewater impact on the Krka River by Daphnid Acute toxicity testing

Vlatka Filipović Marijić<sup>82</sup>, Želimira Cvetković<sup>83</sup>, Camille Boucaud<sup>84</sup>, Ivana Karamatić<sup>82</sup>, Damir Valić<sup>82</sup>, Tomislav Kralj<sup>82</sup>, Tatjana Mijošek<sup>82</sup>, Zuzana Redžović<sup>82</sup>, Vlatka Mikulec<sup>83</sup>, Dušica Ivanković<sup>82</sup>, Zrinka Dragun<sup>82</sup>

**KEYWORDS:** *Daphnia magna*; industrial and municipal wastewaters; Krka National Park; toxic impact

### INTRODUCTION:

In addition to chemical water analyses, assessment of water quality might involve toxicity testing, as a biological tool that reflects toxic impact on aquatic organisms. The commonly used testing organism is crustacean *Daphnia magna* Straus, 1820, which is sensitive to a wide range of contaminants and important consumer in the food chain. Water quality was assessed in the karst Krka River (Croatia), whose lower part was proclaimed national park due to its exceptional natural beauty. Only 2 km upstream from the northern border of the park industrial and municipal wastewaters from the Town of Knin are released in the Krka River without proper purification. Their impact on the river water was assessed at five sites, Krka River source (KRS) as reference location and at four locations downstream of the wastewater impact (industrial (IWW) and municipal (MWW))

---

<sup>82</sup> Ruđer Bošković Institute. Corresponding author: vfilip@irb.hr

<sup>83</sup> Andrija Štampar Teaching Institute of Public Health

<sup>84</sup> University of Côte d'Azur