Rotifer News

A newsletter for rotiferologists throughout the world

Rotifera XVI. Onsite (upper) and Zoom participants (lower left), and a session in progress (lower right). Sources: Maria Špoljar (upper), S.S.S. Sarma (lower left) & Sergio González (lower right).

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In this issue:

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Editorial: *Rotifer News Issue 37 (September 2022)*

The present issue covers the much-awaited Rotifera XVI (5-9, Sept. 2022), which was a great success. This issue presents a summary of the 16th International Rotifer Symposium (IRS) by Maria Špoljar. The IRS XVI attracted more than 150 rotiferologists, the highest number ever recorded from all the previous meetings (see Špoljar´s summary of the meeting in this issue). It is again a great academic stimulus for rotiferologists in Mexico, the country again represented by the largest number of participants. This has been the trend for researchers in Mexico, who greatly appreciate and actively participate in the IRS meetings.

The abstracts of Rotifera XVI have been included in this issue, although the complete Book of Abstracts is also available online from the Conference website. With permission from the Organizers of Rotifera XVI, these abstracts have been added here with the hope that readers who are not members of *Rotifer Family* can have glimpses of contemporary developments in this discipline of science. The original source from the conference website offers additional details such as keynote talks, regular oral and poster presentations.

A few weeks earlier (7-10 August 2022), the prestigious SIL meeting was held in Berlin, which marked the 100th year of this august academic body. During this SIL meeting, works with an emphasis on rotifers represented about 2%, much lower than those of SIL 2021 (see Rotifer News Issue 35, 2022). Probably many rotiferologists preferred to participate in IRS XVI than SIL2022. The contributions (bibliographic citations and abstracts) with emphasis on rotifers from SIL100 have been included in this issue too.

Rotifer News also publishes works in progress or completed projects. In this issue, data of *Aeolosoma* predation on rotifers show the impact of oligochaetes on benthic taxa.

Bibliographic information on recent literature of Rotifera is not included as the present issue is already heavy for online distribution. Photos of social gatherings during the Rotifera XVI will be included in the next issue.

The VRC (Virtual Rotifer Collaboratorium) meetings have been organized and new topics have been brought to the discussion table during these virtual sessions (S. Nandini's report of the VRC will appear in the issue No. 38). Members of the *Rotifer Family,* who have not previously participated, have shown interest to take part in the future VRC meetings. For inclusion in the VRC mailing list kindly contact *nandini@unam.mx*.

Specific units on the **Rotifer News Website** need input. For example, images of identified species are urgently needed for teaching purposes. Please share these. Experts who read *Rotifer News* can also help young researchers confirm the identity of rotifer species. Please send your rotifer images to *Rotifer News*; these will be published and identified, and the information made available as soon as possible.

S.S.S. Sarma
Editor

Exciting moments in Rotifer Research

Summary of Rotifera XVI

The 16th International Rotifer Symposium or Rotifera XVI was held in Zagreb (Croatia) from the 5th to 9th of September 2022, in the Hotel Dubrovnik situated at the main square. Thus, the main cultural highlights and facilities were in the vicinity. Because of demanding global and regional circumstances (COVID pandemic, earthquakes consequences, war in Europe), this meeting was first organised after four instead of three years and was conducted in the hybrid version. Rotifera XVI was held under the auspices of the University of Zagreb (since 1669), Faculty of Science (since 1874) and Department of Biology (since 1876), sponsored by 13 companies/associations, and with the organizing support of the Croatian Ecological Society. The Organizing Committee: Maria Špoljar, Tvrtko Dražina, Ivančica Ternjej, Tea Tomljanović, and Sanja Gottstein were editing the Program Book and Book of Abstracts, and together with Marina Posavec, from the technical organizer agency ProPutuj, enabled planning and executing of the meeting. Scientific Committee members: Jorge Ciros-Perez (Mexico), Steven Declerck (Netherland), Diego Fontaneto (Italy), Holger Herlyn (Germany), Natalia Kuczynska-Kippen, (Poland), David Mark Welch (USA), Evangelia Michaloudi (Greece), Maria Špoljar (Croatia), Elizabeth Walsh (USA), Yufeng Yang (China) reviewed abstracts and were members of committees for oral, poster and photo contest awards during the meeting. It would be impossible to mention all contributions of both committees, but their cooperation and determination contributed to the realization of this conference.

Overall, 151 participants, and six accompanying persons, were present at the conference, 97 onsite and 44 online, respectively. This meeting has gathers scientists from literally every continent, including 25 countries. The most numerous were participants from Mexico (24), USA (20), and Poland (15), eight countries with four to 13 participants (Croatia, Belgium, Germany, Spain, China, Greece, UK, Check Republic) and 14 countries had up to three participants. Altogether, 358 coauthors supported seven invited lectures, 64 oral and 77 poster presentations. In the ratio of students *vs* PhD and professors, one-third of the participants belong to the new arrival young scientists and give us hope in those who will cherish rotifer topics in the future. The conference was enriched with two special sessions and three workshops, all successfully conducted and hopefully helped in the further activities of the Rotifer family. Among scientific achievements, experimental genomic research gains significant results, of which the discovery of meiosis in the Bdelloidea is outstanding. Compared to numerous brilliant studies conducted with *Brachionus*, those related to the diversity and ecology of rotifers in different habitats noticed a

slight decrease, particularly in oral presentations. However, there were also studies which underlined rotifer impact in the ecosystem stoichiometry and rotifer assemblage in many interesting habitats, e.g. ponds, karst lakes, ponds and sinkholes, mats from Antarctica, the fire burned soil etc. In the Preface of the Hydrobiologia Special Issue dedicated to the studies from Rotifera XVI would be a more detail survey for each session from the meeting.

The social aspect of the meeting was marked by the opening and closing ceremony and mid- and post-conference excursions. The opening ceremony in the old Upper Town started with the academic anthem *Gaudeamus igitur*, a speech by Maria Špoljar, the conference organizer, dedicated to the great motive of our small circle of the great people, and Dean of Faculty of Science, Mirko Planinić (see cover photo). They highlighted the importance of the application of scientific results for the progress of society and environment. The further program continued with the songs from main regions in Croatia performed by the academic choir "Ivan Goran Kovačić". Mid-conference excursion revealed the karst hydro-system of the Plitvice Lakes National Park, and the post-conference excursion on the Krk Island was more dedicated to the cultural and natural features of the Mediterranean region of Croatia. At the Closing Dinner in the Down Town modern building on the roof terrace, a special time was dedicated for the awards to the young scientists in the categories of oral (1st Katrin Kiemel, 2nd Victoria Carla Moris, 3rd Patrick D. Brown, and a special award to Tomatake Wada for enhancing in the oral presentation), and poster presentations (1st Julia R. Geppert, 2nd Alexandros Vasilikopoulos, 3rd Mayra Carolina Reyes-Santillan) and in photo contest (1st Julia R. Geppert, 2nd Christopher Wilson, 3rd Vicente Franch).

We congratulate all awarded students and their mentors, all participants, and all unmentioned persons involved in the organization and providing the Rotifera XVI meeting. Special thanks are due to Elizabeth Walsh, organizer of Rotifer XV, for numerous useful suggestions and support.

I sincerely hope that scientific aspects of the Rotifera XVI confirm our determination in the further research of our fascinating rotifer creatures and bring us closer to their diversity, ecology, genomics and evolutionary aspects. In the social aspects, I would like this conference's spirit to take longer.

Until now, Organizing Committee, thank you for all the beautiful words related to the meeting. Also, we wish good luck for the next host of Rotifera XVII.

Maria Špoljar
Organizer, Rotifer XVI

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Pioneers of Rotifer Research

The pioneer of rotifer research in Argentina:

Santiago Raul Olivier (1923-2008)

Dr. Santiago Raul Olivier was born in La Plata (Province of Buenos Aires, Argentina), a city known for its prestigious School of Natural Sciences and Museum of University Nacional de la Plata (UNLP). He was a well-known biologist, an enthusiastic teacher and researcher.

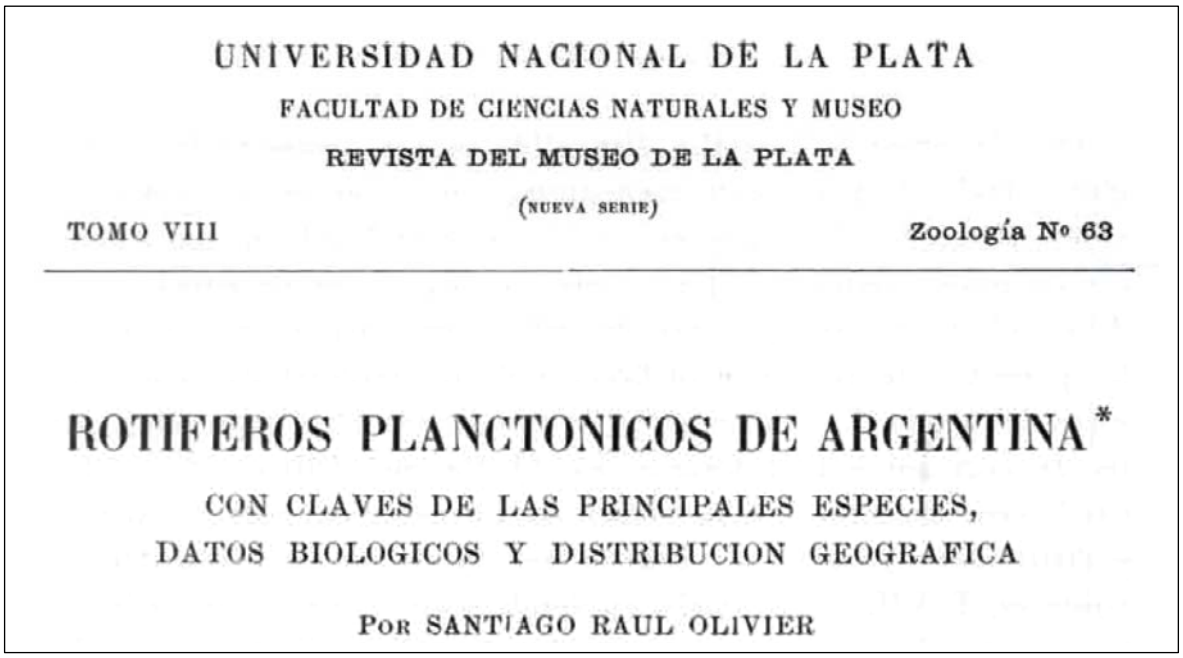
In the first half of the 20th century, only a few foreign scientists were interested in the study of Argentinian inland water rotifers (Wierzejski, Daday, Alhstrom, Thomasson, among them). Dr. Santiago R. Olivier began his studies on rotifers after its doctoral thesis (University of La Plata, 1949) studying seasonal variations of rotifers of the Chascomus Lake, an important body of water in the Province of Buenos Aires. Later, in the framework of limnological research, he studied the rotifers of different water bodies, La Salada Lake, Vitel Lake, among others, mainly shallow lakes, some of them little known until then (Olivier 1952, 1955, 1961). This allowed the knowledge of the taxonomic diversity of rotifers, their seasonal variations, at the group and species level, and their relationship with environmental parameters (Vitel Lake) or the horizontal distribution (La Salada Lake). Besides, his study in the Vitel Lake provided a lot of information on the physical, chemical and biological features of the environment, including detailed information on the macrophytes present and the ichthyofauna.

He published in 1961 an analysis the rotifers composition of more than 50 Pampean shallow lakes, as indicators in limnology, focusing on the species of the genera *Brachionus* and *Keratella* and their presence associated with different ranges of salinity and alkalinity.

In 1965, he published his paper Planktonic rotifers of Argentina, Revista del Museo de la Plata (Fig. 1). This publication was very important for the following generations of students and researchers who would start studying rotifers. It presents a key to families, genera and species of rotifers known up to that time throughout the country. Includes descriptions of more important morphological characters of the taxa, besides data on the geographic distribution throughout the Argentine territory.

His studies on rotifers lasted little more than a decade, but he left an important guide for those interested in the knowledge of Rotifera, which did not have a key to the species of the country, and particularly in the native language.

He devoted himself entirely to marine biology over time, donating much of his library on inland waters to Institute of Limnology, Santo Tome-Santa Fe. He was the first director of the Institute of Marine Biology of Mar del Plata. He was always interested in the sociological and political aspects of environmental problems, was Chair



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ROTIFEROS PLANCTONICOS DE ARGENTINA*

CON CLAVES DE LAS PRINCIPALES ESPECIES,
DATOS BIOLOGICOS Y DISTRIBUCION GEOGRAFICA

Por SANTIAGO RAUL OLIVIER

Fig. 1 Source (full text) https://publicaciones.fcnym.unlp.edu.ar/rmlp/article/view/1815

Professor of "Ecology and Society", UN Environment Programme Advisor, 1980-1984. He was author of the books as “Ecology and underdevelopment in Latin America" (1986). The scientific production of Dr. R. Olivier reveals the personality of a curious scientist interested in the field of Ecology. In the last years of his long life, he continued to write and publish with great passion.

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Invited Lectures on Rotifers

Rotifer Cultures, Ecological Roles and Applicational Values

S. Nandini and S.S.S. Sarma visited the Freshwater Biology Regional Centre (FBRC), Hyderabad, India during the month of August 2022 and delivered invited talks on rotifers, with reference to their culture systems, ecological importance and applicational values in the current scenario (Figs. 1-4).

India has more than 400 species of rotifers which have been documented by various pioneering workers. B.K. Sharma and Sumita Sharma have made significant contributions to the rotifer fauna of India during the past few decades. Yet, other aspects of rotifer research such as the applications, ecological values and ecosystem services have not received considerable attention. The recent lectures by Nandini and Sarma provide broad knowledge on the ecological importance of the rotifers and their role in ecosystem services to society.

During lectures, thought provoking ideas have been shared and discussed on the culture systems of rotifers, ecological assessment and their applications. It was an immense nourishment to the researchers and scholars at the Freshwater Biology Regional Centre, Hyderabad. We are very grateful to S. Nandini and S.S.S. Sarma. I thank them on behalf of Director, Zoological Survey of India and Officer in Charge, Freshwater Biology Regional Centre, Hyderabad, India and I look forward for more visits to the centre in the near future.

Fig. 1. Lectures in progress

Fig. 2. Lectures in progress

Fig. 3. Visit to the Museum of FBRC

Fig. 4. Participants of the special talks

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Notes and News

Sessile rotifers in a green pool

During a field sampling (January 2022) from the Valle de Bravo reservoir (State of Mexico, Mexico), water hyacinth (*Eichhornia crassipes*) was found scattered on the shore and floating in the water body (Fig. 1). We collected roots (20 cm long) of water hyacinth and examined them under microscope. Four sessile rotifers were identified: *Beauchampia crucigere* (Dutrocet, 1812), *Floscularia ringens* (Linnaeus, 1758) (Fig. 2), *Limnias ceratophylli* Schrank, 1803 and *Stephanoceros millsii* (Kellicott, 1885) (see *https://youtu.be/6DR_g3vFXB8*).

Valle de Bravo reservoir is meso-eutrophic with several cyanobacterial genera such as *Microcystis* and *Dolichospermum* some of which are toxic (Gaytán-Herrera et al., 2011; Nandini et al., 2019). Previous studies from this waterbody have mostly reported free-living rotifer species

Fig. 1. Valle de Bravo Reservoir (Mexico). Photos: Rodríguez Velázquez Delfino

(Figueroa-Sánchez et al., 2014). The diversity of sessile rotifers from this waterbody, based on a single collection shows high potential to study the diversity of sessile rotifers. Further studies involving spatial and temporal sampling would certainly enhance understanding on the tolerance capacities of sessile rotifers living and reproducing under poor water quality conditions.

Fig. 2. *Floscularia ringens*

Authors are grateful to Dr. Miloslav Devetter for revising the text. MAFS thanks COMECyT-CAT2021-0144.

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Rotifers and SIL 2022

Rotifers in the SIL100 were under-represented.

*Abstracts with permission to reproduce and Bibliographic details of works containing **Rotifer*** from the 36th Congress of the International Society of Limnology (SIL) (7–10 August 2022, Berlin) are included here. The original abstract numbering has been retained for easy reference.*

ON287 Mysterious ciliates: seasonally recurrent and yet hard to predict

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ON167 Effect of anionic surfactant on the demography of Plationus patulus (Rotifera)

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Aquatic environments are continuously altered by different types of pollutants, many of them are of xenobiotic origin and among those that most affect water bodies are antibiotics, microplastics and surfactants. This last group is highly diverse since it is found in a large number of industrial products of daily use. Cleaning and disinfection properties of surfactants are enhanced by additives such as wetting agents, adherents and emulsifiers. Anionic surfactants are generally of synthetic origin such as sodium dodecyl sulphate (SDS). SDS has been found to have negative ecological effects on aquatic organisms. Currently anionic surfactants in Mexico have scarce regulation. Therefore, the aim of this work was to investigate the effects of SDS on a common rotifer, Plationus patulus. We tested the effect of different concentrations (1.25, 2.5, 5, 10 and 20 mg/L) of SDS on population growth and life table demography of rotifers. Our resulted showed that with increase in the concentrations of SDS, there was a decrease in the survival and fecundity rotifers. Population growth curves showed decreased abundances with increasing concentration of SDS.

ON311 Zooplankton functional groups as water quality indicators: application to reservoirs

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ON343 Impacts of an invasive omnivore shrimp on plankton communities: a mesocosm experiment from a food web perspective

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ON203 Shedding light on zooplankton diversity from the Congo River Basin

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ON397 How nitrogen and phosphorus supply to nutrient-limited phytoplankton communities affects zooplankton growth: testing stoichiometric and co-limitation theory across trophic levels

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ON342 Feeding efficiency of the exotic copepod Mesocyclops pehpeiensis in the presence of the native species Mesocyclops longisetus curvatus

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The introduction of invasive species is one of the four main causes of biodiversity loss. In Mexico the exotic species of copepods include Thermocyclops crassus, Mesocyclops aspericornis, Mesocyclops thermocyclopoides and Mesocyclops pehpeiensis. Here we tested the presence of the invasive Mesocyclops pehpeiensison the feeding behavior of an analogous native species, M. longisetus. We hypothesized that the invasive species would use the food resources more efficiently than the native species. Food preferences and consumption rates of M. pehpeiensis and M. longisetus curvatus were determined, separately and together in the same environment, prey items consisting of five cladocerans and five rotifers. The copepod predators were pre-starved for different periods (2 h, 4 h, and 10 h) prior to offering prey items. When tested alone, after a 2h starvation, M. pehpeiensis consumed prey biomass of 8.55 µg/ind. and after 4h starvation M. longisetus curvatus consumed 17.3 µg/ind. However, in mixed culture, after 4h starvation, both the predator species consumed 9.0 µg/ind. For the copepod species, optimal feeding rates were observed following two or four hours of starvation. Results have been discussed with emphasis on factors promoting the widespread distribution of the invasive M. pehpeiensis in Mexico.

ON291 Mussels can facilitate the restoration of eutrophic waters by improving water clarity, enhancing macrophyte growth and changing plankton community

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OR077 Effect of copper (Cu) and temperature on the feeding behavior of the invasive (Mesocyclops pehpeiensis) and native (Mesocyclops longisetus and Microcyclops dubitabilis) copepods (Crustacea: Copepoda)

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Deterioration of water quality due to metal contamination is increasing; copper (Cu), is one of the most important heavy metal pollutants in Mexico. While rotifers and cladocerans are frequently used in ecotoxicological bioassays, copepods are rarely used. Here we compared the effect of copper on the feeding behavior of the invasive copepod Mesocyclops pehpeiensisand the native species, Mesocyclops longisetusand Microcyclops dubitabilis. Weoffered 6 prey species (rotifers and cladocerans) at two copper concentrations and three different temperatures (18, 23 and 28°C). We observed significant differences in the prey preference and consumption by the three copepod species depending on the Cu concentration and temperature level. All test copepods showed a greater preference for the prey Moina macrocopa (Cladocera) and Brachionus calyciflorus (Rotifera). The copepods selected more prey types in the controls and at lower Cu concentrations. The results have been analyzed in relation to the effect of copper on prey selection by native and invasive copepod predators.

OR035 Effect of microplastics on the population growth of rotifers (Rotifera)

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Many epicontinental aquatic ecosystems are constantly exposed to polluted wastewaters or partially treated industrial and domestic effluents. Currently, microplastics have a great relevance to ecosystem health due to their minute size and high abundances, both of which allow them to be ingested by many filter-feeding aquatic animals at different trophic levels. Microplastics in different size ranges (100 nm to 5 mm) can affect the demography of herbivorous zooplankton species in different ways, such as clogging the filtering apparatus (mechanical interference) or reaching the gut through ingestion. In this work we evaluated the demographic responses of two herbivorous rotifers of different sizes Plationus patulus (160 µm) and Brachionus havanaensis (120 µm) that were exposed to one concentration (5 mg L-1) of polystyrene microspheres 30 µm in diameter together with the green alga Chlorella as food. Compared to controls, algal food containing microplastics had significant effects on the selected demographic variables. Results were discussed in relation to the growing threat from microplastics to filter-feeders in freshwater ecosystems.

OR003 Effect of crustacean (cladocerans and copepods) conditioned medium on the algal food intake and filtration rates of the rotifer Brachionus havanaensis (Rotifera)

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Competitive interactions of herbivorous crustaceans such as cladocerans and copepods with

rotifers, indicate that the former group seems to have an advantage over the latter not only mechanically but also chemically by releasing compounds into the medium. By both, mechanical and chemical interference, crustaceans dominate in natural waterbodies. Infochemicals released by the crustaceans may interfere with other ecological and physiological processes of rotifers such as feeding and filtration rates, which in the short and long term influence the organisms' fitness. To test whether crustacean allelochemicals can affect shortterm and long-term feeding process of rotifers, we measured the feeding and filtration rates of the rotifer Brachionus havanaensis using the conditioned medium obtained separately from the cladocerans Ceriodaphnia dubia and Moina macrocopa, and the copepod Arctodiaptomus dorsalis (females and males, separately). For feeding rotifers, we used the single-celled green alga Chlorella vulgaris at a concentration of 0.5x106 cell/ml. The rotifers were pre-starved for 30 min, and then fed on alga for 1 h in the presence or absence of crustacean conditioned medium. The response of rotifer feeding and filtration rates differed significantly depending on the source (cladocerans or copepods) of the conditioned medium.

OR037 Zooplankton indicators of Water quality in three reservoirs in the State of Mexico

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The Cutzamala system supplies water to millions of people in Mexico City. Among the important sources of water for this hydropower system are the two reservoirs, Valle de Bravo and Villa Victoria, both in the State of Mexico. The Madín reservoir, also in the State of Mexico, provides drinking water to several thousands of residents in northern suburbs of Mexico City. In this study we analyzed the water quality based on zooplankton density and diversity from six sites of Valle de Bravo, Villa Victoria and Madín. Eighty litres of water were filtered at each site using a 50µm mesh and preserved on the spot using formalin at 4%. Standard physical and chemical variables of the reservoirs water were also estimated. Samples will continue to be collected during September 2022 to August 2023. Our analysis indicated that the Villa Victoria reservoir had the highest density of zooplankton. The dominant rotifers were included various species of Trichocerca, Polyarthra vulgaris, Kellicottia bostoniensis and Pomholyx sulcataand the cladocerans Daphnia laevis and Bosmina longirostris. In the Valle de Bravo reservoir we observed dense blooms of cyanobacteria, especially Microcystis, Oscillatoria and Dolichospermum; the rotifers

Polyarthra vulgaris and the cladoceranChydorus sp. were dominant. In the influent and effluent of the water treatment plant of Madín reservoir we observed low densities of cyclopoid and calanoid copepods and few species of rotifers, mostly brachionids. The zooplankton analyses showed that the water in the treatment plants of Villa Victoria and Madín reservoirs are mesotrophic with low saprobic indices.

OR002 Zooplankton abundance and biomass in shallow eutrophic Yeniçağa Lake (Bolu, Turkey)

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EP178 Zooplankton Communities as Bioindicators of Water Quality in Lake Palacpaquen and Lake Pandin, San Pablo City, Philippines

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EP167 Morphometric variations of rotifers in a high altitude reservoir in Central Mexico

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Studies on rotifers from Mexico have mainly focused on species richness and abundance. Biotic factors such as predation and abiotic factors such as temperature and pH cause morphometric variability of rotifers. There are only a few studies that mention the morphometry of rotifers mainly based on laboratory experiments between predator and prey interactions. In this study, geometric morphometry was employed to characterize and quantify the lorica variations in the selected rotifer species of the genera Trichotria, Testudinella, Trichocerca, Mytilina, Lecane and Keratella. Rotifers used to estimate the geometric morphology came from the high altitude (2850 m.a.s.l.) Llano reservoir, located in central Mexico. Both Mytilina ventralis (180-260 µm) and Trichotria pocillum (190-265 µm) showed marked variations in the lorica lengths. Morphometric variations of chosen rotifer species were interpreted in relation to temperature, pH, dissolved oxygen, conductivity, chlorophyll a, and nutrient levels.

EP101 An empirical assessment of the threat of suspended microplastics to aquatic food webs in a neotropical freshwater wetland

Carlos López[1], Luz Marina Soto[2], Vanessa Acosta[3], Luciana Gomes-Barbosa[4], Georgia Stamou[5], Evangelia Michaloudi[6], Maciej Karpowicz[7], Miriam Steinitz-Kannan[8]
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EP030 Temperature-mediated diet preference in the invasive crustacean Limnomysis benedeni

Varsha Rani1, Ádám Fierpasz[1], Zsófia Horváth[1], Péter Borza[1], Pavel Kratina[2], Károly Pálffy[1], Csaba F. Vad[1]
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EP145 Effects of cyanotoxins on the demography of successive generations of the rotifer *Brachionus calyciflorus.*

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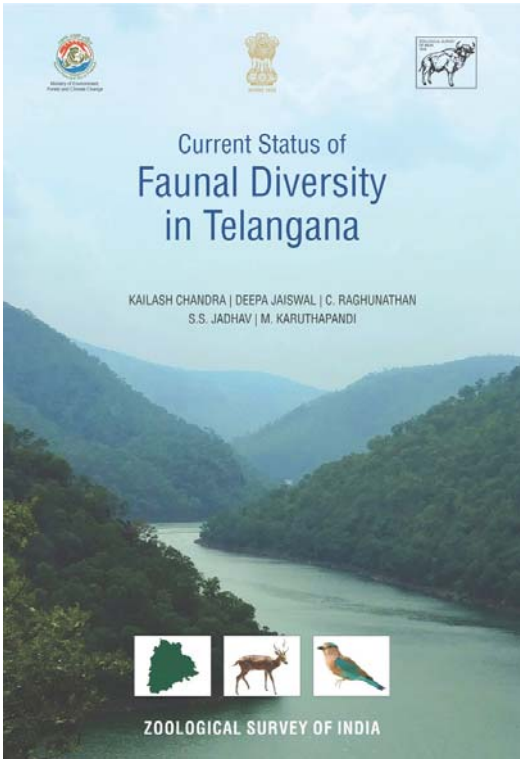
[1]FES Iztacala, Universidad Nacional Autónoma de México, Tlanepantla, Mexico.

Rise in aquatic pollution due to an increase in anthropogenic activities has led to eutrophication, and proliferation of cyanobacteria. These cyanobacteria have several adverse effects on zooplankton, often due to the production of toxic secondary metabolites such as cyanotoxins. Many zooplankton species have developed physiological and behavioural strategies that allow them to coexist with cyanobacteria. We evaluated the acute and chronic effects of different concentrations of the cyanobacterial crude extract from the Valle de Bravo Reservoir on the rotifer *Brachionus calyciflorus* isolated from a local water body. We filtered 100 l of the reservoir water and estimated the diversity and density of cyanobacteria. The crude extract, after five cycles of freezing, thawing and sonication at 14 MHz, was filtered and the microcystin concentration quantified based on ELISA. Acute toxicity tests were conducted based on 24h mortality. Chronic toxicity tests (life table experiments) of *B. calyciflorus* were conducted for two consecutive generations (F0 and F1) at three sublethal concentrations (0.238, 0.476 and 0.952 µg/L) based

on LC50 data (2.56 µg/L). The field sample was dominated by *Woronichinia naegeliana*. Population growth rates of the F_0 *B. calyciflorus* exposed to sublethal concentrations of the cyanotoxins ranged from 0.15 to 0.24 and for the F_1 from 0.16 to 0.42. Our results further showed that the second generation exposed to cyanotoxins had a better fitness than the F_0.

Regional Guides on Rotifera



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Contents

and other chapters……

Chapter 05

Rotifera

JAYASHREE TILAK[1] and KARUTHAPANDI, M.[2]

Abstract: Eighty species of rotifers belonging to 29 genera and 18 families were reported from Telangana. The highest number of species belonging to family Brachionidae and Lecanidae had 20 species each, both constitute 50% rotifers of Telangana. Most of rotifer faunal documentation is in and around Hyderabad city, not much extensive studies from the state of Telangana. It needs systematic rotifers exploration, because most of times it has been encountered while studying zooplankton diversity assessment and following old literatures and lack taxonomic knowledge about species leads to misidentified.

Keywords: Rotifers, Diversity, Telangana

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Rotifers and *Aeolosoma*

Rotifers attacked by *Aeolosoma hemprichi* Ehrenberg, 1831: observations of feeding behavior

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Abstract. Interaction between rotifers species with *Aeolosoma hemprichi* is poorly understood. Also their feeding habits and biological interaction. Therefore, the present study had as a main goal to perform observations on the in vitro feeding behavior. The organisms were cultured with a photoperiod of 16h of light and 8h of darkness at a temperature of 25ºC in a bioclimatic chamber; they were fed on the alga *Nannochloris oculata* and lemon tree leaves, 1cm x 1cm, in reconstituted water as proposed by the United States Environmental Protection Agency (USEPA). Later on, inter-specific competition experiments were carried out with three rotifer species: *Asplanchna brightwellii*, *Brachionus calyciflorus* and *Lecane quadridentata*, cultured under the same laboratory conditions. Videos of the feeding behavior over the competition experiments were recorded. The authors report for *A. hemprichi*, the capacity to decrease the intrinsic growth of *B. calyciflorus*; on the contrary, it does not achieve so when it competes with *L. quadridentata*. While, *A. hemprichi* and *A. brightwellii* prey on one another, there are videos, as well as step-by-step schemas of this voracious behavior.

Keywords: biological interactions, detritus pellets, zooplankton.

Introduction

Rotifers are small (50-2000 µm) and predominantly live in freshwater, but in saltwater as well; they feed on detritus, bacteria, algae, protozoans and other rotifers. Rotifers are important because they can achieve high population densities (> 5,000 individual/L) and they are a critical link between algal production and higher trophic levels (Beres *et al*. 2005). While, Oligochaeta and genus *Aeolosoma*, represent the family

Aphanoneura and contain the most primitive members of oligochaetes (Nelson 1906). Aeolosomatidae are cosmopolitan worms with apparently simple organization, living mainly in freshwater ecosystems occurring mostly in limnetic habitats, are important as a detritus feeder (Bunke 1988). Both invertebrates usually living in freshwater ecosystems and their biological interactions in zooplankton communities are evident, though poorly studied. Rotifers and Oligochaeta, it is probably to compete for the similar resource, and niche. Consequently, the exited in both a dynamics predator – prey having a direct effect removing one species or population, including the modification of the predator or prey behavior, life history and morphological traits (Lass & Spaak 2003). As a consequence, for example, as the authors know benthic rotifers interact with other benthic species, potentially specimens of Oligochaete, whereas planktonic rotifers occasionally interact with benthic species. Indeed, competition for a common resource or requirement that is actually, or potentially, limited, is known to take place between individuals of the same species (intraspecific competition) or between members of a number of species at the same trophic level: interspecific competition (Han 2019). For this reason, species competition is very complex however an important ecological indicator in the trophic chain: for example, rotifers are constantly predated by other organisms such as fish, copepods and also rotifers such as *Asplanchna brightwellii* or *A. sieboldi*, affecting the rotifers population rate, but increasing predator species. Definitely, studies on predation demonstrate for copepod *Diaptomus pallidus* increased ingestion rates 5.5 – 6-2 greater with rotifer (*Synchaeta oblonga*) as live food than on algae (*Cryptomonas reflexa*), in fact, the survival and reproduction of the copepod were significantly enhanced. Interestingly, as an evolutionary response to attacked, some rotifers have developed specific strategies to defend and escape from some predators. For example: a) rotifers induce formations of spines and polymorphism (Gilbert & Williamson 1978; Gilbert 1985); b) other mechanisms are trade-off in sexual and asexual reproduction in rotifers mediated by increased of population or crowding (Sarma *et al*. 2002), c) and, rotifers (*Keratella* spp.) sometimes displayed an escape response to some predators (Gilbert & Kirk 1988). In resume, the importance to study on rotifers interspecific competition it is priority, because ecotoxicology applications, and evolutionary behavior. Owing to this, we study the biological interactions and discuss the ecological relevance of the interaction of rotifers with oligochaetae, and the effect in the growth rate of rotifers in the presence of *Aeolosoma hemprichi*. Moreover, we observed and described stages in all the biological behavior, especially in the formation of detritus and the conduct of predator and prey.

Material and methods

Culture of rotifers and oligochaete

Rotifers *Brachionus calyciflorus* and *Lecane quadridentata* were cultured

according to the protocol by Pérez-Legaspi & Rico-Martínez (1998). They were placed in Petri dishes with reconstituted medium proposed by EPA (U.S Environmental Protection Agency 1985) and were fed on green alga *Nannochloris oculata* (strain LB2194 from the collection of the University of Texas) cultured in Bold's medium (Nichols 1973). *Asplanchna brigthwellii* was cultured in Petri dishes and was fed on *B. calyciflorus*. The rotifer species were identified with Koste (1978) keys and are part of the strains on the toxicology laboratory of the Autonomous University of Aguascalientes. Oligochaete *Aeolosoma hemprichi* was isolated from a waterbody called *La Mezquitera* (21.917749 N; -102.316968 W), located at the premises of the university above. Specimens were identified with Brinkhurst's (1971) taxonomic keys, they were also cultured in Petri dishes and fed on green alga *N*. *oculata* and additionally on 1-cm^2 pieces of lemon leaves. All the organisms were placed in a bioclimatic chamber with photoperiods of 16:8 (light/darkness), at a temperature of 25°C ± 2°C. All the experiments with rotifers used newborn rotifers younger than 24 hours. *A. brightwellii* females were adults taken from the stock of cultures fed on *B. calyciflorus*. In the case of oligochaete *A*. *hemprichi*, organisms recently detached from their mothers and small-size ones were used, as this oligochaete does not provide asexual eggs to control hatching, as it occurs with rotifers. The foregoing on the basis of reports by Nelson (1906), who mentions that the reproduction of *Aeolosoma hemprichi*, is described as an asexual strategy. The latter consists in a process of fission or budding, by means of which the young individual is constricted off from the posterior portion of the parent; this process often takes place so rapidly that chains of individuals are formed, representing three or more generations. This process is continuous over the life of the individual and probably ample suffices, as far as numbers are concerned, to ensure the survival of the species.

In vitro biological interactions

Brachionus calyciflorus (BC) versus *Aeolosoma hemprichi* (AH): both species were placed together at various proportions, as follows: 1 AH female and 5 BC females; 2 AH females and 5 BC females; 3 AH females and 5 BC females; and, 4 AH females and 5 BC females. The final volume in all the experiments was 2 ml and were fed on the alga *N. oculata*, once at the beginning of the experiments at a concentration of $5x10^6$ cells/ml. They were incubated at a temperature of 25ºC for 4 days with a photoperiod of 16 h light and 8 h darkness. At the end of the experiment, the live organisms of each species were counted. Additionally, in this experiment the number of detritus pellets formed by the oligochaetes and the number of oligochaetes located in these pellets were counted, subsequently, we development an experiment of detritus pellets formation: 10, 20, 30 and 40

A. *hemprichi*, in a volume of 1 ml, with 10 *B. calyciflorus*, 5X10^6 cells/ml of *N. oculata* as a feed. Six replicas were carried out. At the end of experiments, we counted detritus pellets and organisms located into detritus pellets.

Lecane quadridentata (LQ) versus *A. hemprichi* (AH): both species were placed together in the following proportions: 5 AH females and 10 LQ females. The final volume was 2 ml and were fed on *N. oculata* once at the beginning of the experiment at a concentration of 5x10^6 cells/ml. They were incubated at 25°C for 4 days with a photoperiod of 16 h light and 8 h darkness. At the end of each experiment the living organisms of each species were counted. Six replicas were carried out.

Asplanchna brigthwellii (AB) versus *A. hemprichi* (AH): both species were placed in the following proportion: 5 AH females and 1 AB female, the final volume was 2 ml and were fed on alga *N. oculata* once at the beginning of the experiment at a concentration of 5x10^6 cel/m, and 10 *Brachionus calyciflorus* were added at 0 h. They were incubated at a temperature of 20°C, 25°C with a photoperiod of 16h light and 8h darkness. At the end of each experiment, the total number of rotifers was counted. Eight replicas were carried out.

Statistical analyses were performed in Software Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA). The averages and standard deviation of the experiments are presented; additionally, a one-way ANOVA analysis was carried out with a Duncan post hoc comparison to find out the statistical differences between experiments.

Videotapes

The experiments were videotaped: when rotifer *A. brigthwellii* tried to eat oligochaete *Aeolosoma hemprichi*, and when oligochaete *A. hemprichi* interacts with *L. quadridentata*, and the formation of detritus pellets in the interaction *B. calyciflorus* versus *A. hemprichi*; using a microscope Leica DMLS WITH camera Infinity Capture 4.6.0 Software (Lumenera Co., Ottawa, ON, Canada). All the videos were edited with Movie Maker Windows 2.6 saved as AVI, 30.0 Mbps, 720 x 480 pixels, aspect ratio 4:3 and 30 photograms a second.

Results

We observed some biological interaction behavior rotifers with A. *hemprichi* for the *in vitro* experiments, where: *A. hemprichi* and *A. brightwellii* are top predators and compete with one another. Whereas, *L. quadridentata* and *B. calyciflorus* as primary consumer on *N. oculata*; while *A. brightwellii* it is a secondary consumer on *B. calyciflorus*. Figure 1.

In the biological interaction experiments it was observed that *L*. *quadridentata* does not reduce its population due to the presence of *A*. *hemprichi* (Figure 2), in comparison with *B. calyciflorus*, which have an extraordinary decrease in its population due to the presence of *A*. *hemprichi* (Figure 2), even in the presence of one oligochaete. We observed a drastically decrease in the population of *B. calyciflorus* when two, three and four oligochaetes were added. In the experiments with

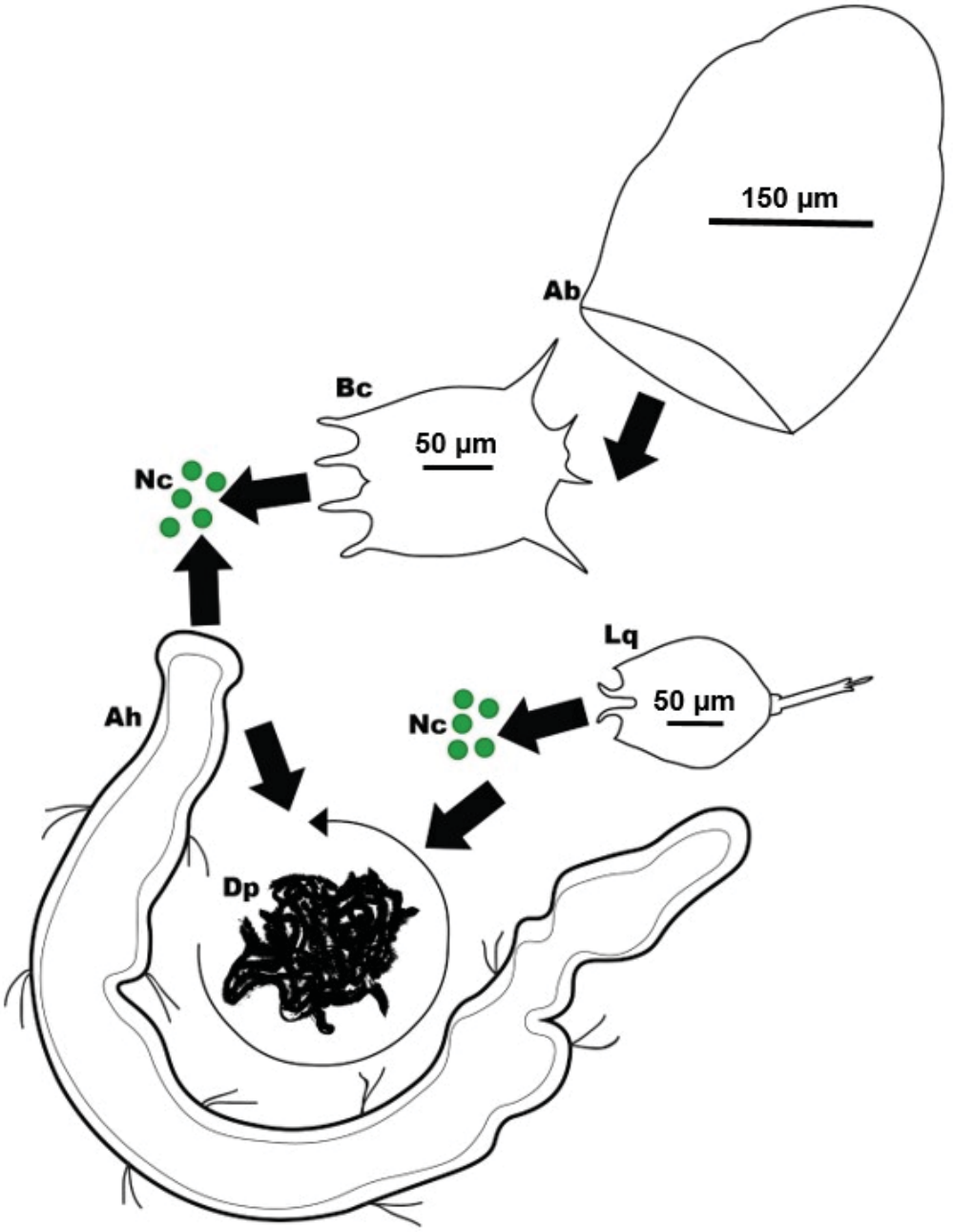

Figure 1. Conceptual model of a trophic interaction with rotifers, oligochaete and algae. Ah = *Aeolosoma hemprichi* as a tertiary consumer; Ab = *Asplanchna brightwellii* as a second consumer; Bc = *Brachionus calyciflorus*, and Lq = *Lecane quadridentata* both rotifers as a primary consumer, Nc = *Nannochloropsis oculata* as a primary producer, and Dp = Detritus pellets.

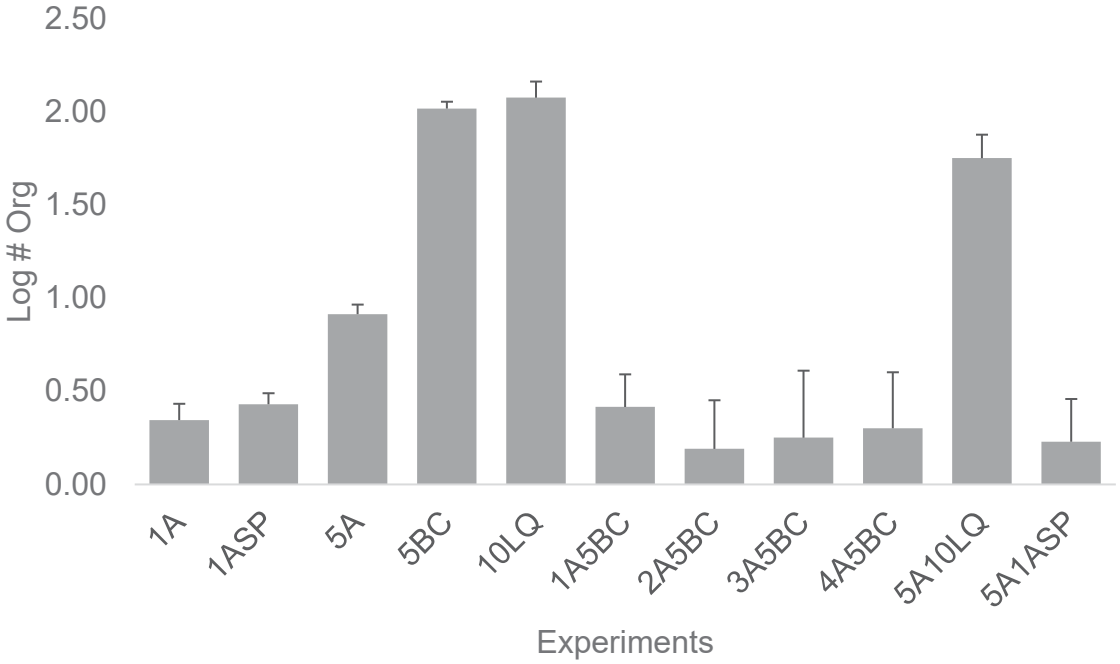

Figure 2. Effect on growth population (total organism at the end of experiments expressed as Log) of rotifers interacted with *Aeolosoma hemprichi*. Values are means ± standard deviations.

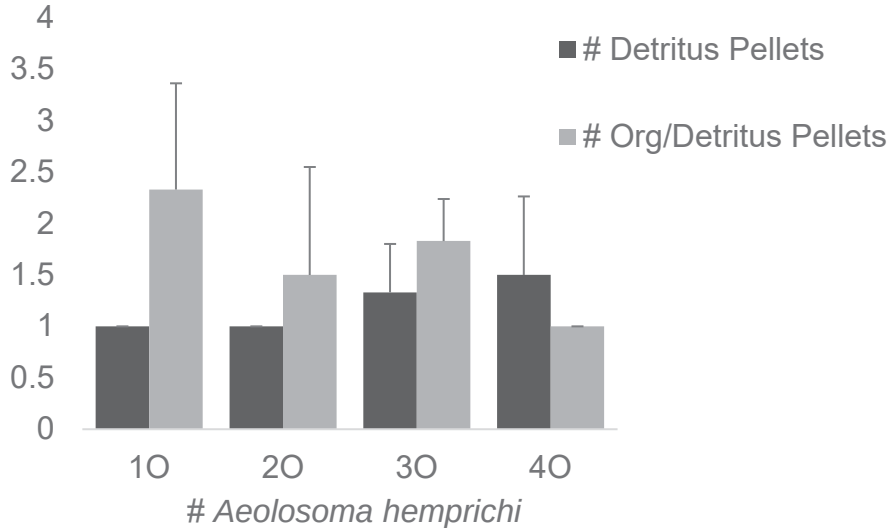

Figure 3. Detritus pellets formation by *Aeolosoma hemprichi*, at different density of organisms feeding with 10 *Brachionus calyciflorus*, and $5X10^6$ cells/ml of *Nannochloropsis oculata*. Values are means ± standard deviations.

A. brightwellii in which 5 oligochaetes were added, there was a decrease in the population of rotifers (Figure 2), but not similar as observed for *B. calyciflorus*. In all the experiments the oligochaetes population variations were non-significant, to be precise, they did not show significant increments while living together with all the rotifer species.

The effect of the number of organisms at the end of the interaction between rotifer predator *A. brigthwellii* and oligochaete *A. hemprichi* are: *A. brightwellii* grows well at a temperature of 25°C over a 48-hour period feeding on *Brachionus calyciflorus*. When five oligochaetes are added, the population growth of *A. hemprichi* is highly variable and not statistically significant differences compared with the corresponding controls. The interaction experiments between *A. brightwellii* and *A. hemprichi*, as well as those with both rotifers, were videotaped to document the predation process between both organisms and explain the variability of the obtained data as following.

Observation of feeding behavior of A. hemprichi in presence of rotifers.

First observation. The process to make detritus pellets is to move in circles and stretching the body of the oligochaete, then it curls and thus it compacts the detritus and produces the pellets with a stick mucilage (not characterized for this work) that its body exudes and which serves as a trap to catch other rotifers that are still alive (video range minutes; 00:00:00 – 00:00:14). On average, it makes 1-1.5 pellets of detritus in the biologic interaction experiment with *B. calyciflorus*, in which on average it was noticed from 1 to 2.83 oligochaetes living in detritus pellets; which indicates that the fewer the oligochaetes, the larger the number of detritus pellets (Figure 3).

Link to see the video:
https://youtu.be/oRVHVeqsGxo.

Second observation. *A. hemprichi* cultures groups of oligochaetes were observed attacking rotifers of the species *L*. *quadridentata*, in which one notices how they interacted in this pack of rotifers and oligochaetes (video range minutes; 00:00:15 – 00:00:27). The interaction between species of *L. quadridentata* and *A. hemprichi* seems to be good, as in the results a decrease in the population of *L. quadridentata* when it interacted with *A. hemprichi* was not observed.

Link to see the video:
https://youtu.be/CriwbpPqGx8.

Third observation. *A. brigthwellii* expect to eat oligochaetes unsuccessfully, even after a number of attempts as the oligochaete curls to avoid being eaten; this curling process suggests a defense mechanism of the oligochaete (Figure 4). On the contrary, rotifer *A. brightwellii* has an aggressive voracious behavior. As response to the rotifer attack, the oligochaete curls but now inside the rotifers body (video range minutes; 00:00:28 – 00:01:03) as a response mechanism. This curling makes the rotifer paralyze for moments, which allows the oligochaete to move into the rotifer's body in order to brush its mucilaginous in external lorica of the body and damage it with the serrated bristles it has (video range minutes; 00:00:36 – 00:01:03). This curling is identical to the one it makes when it

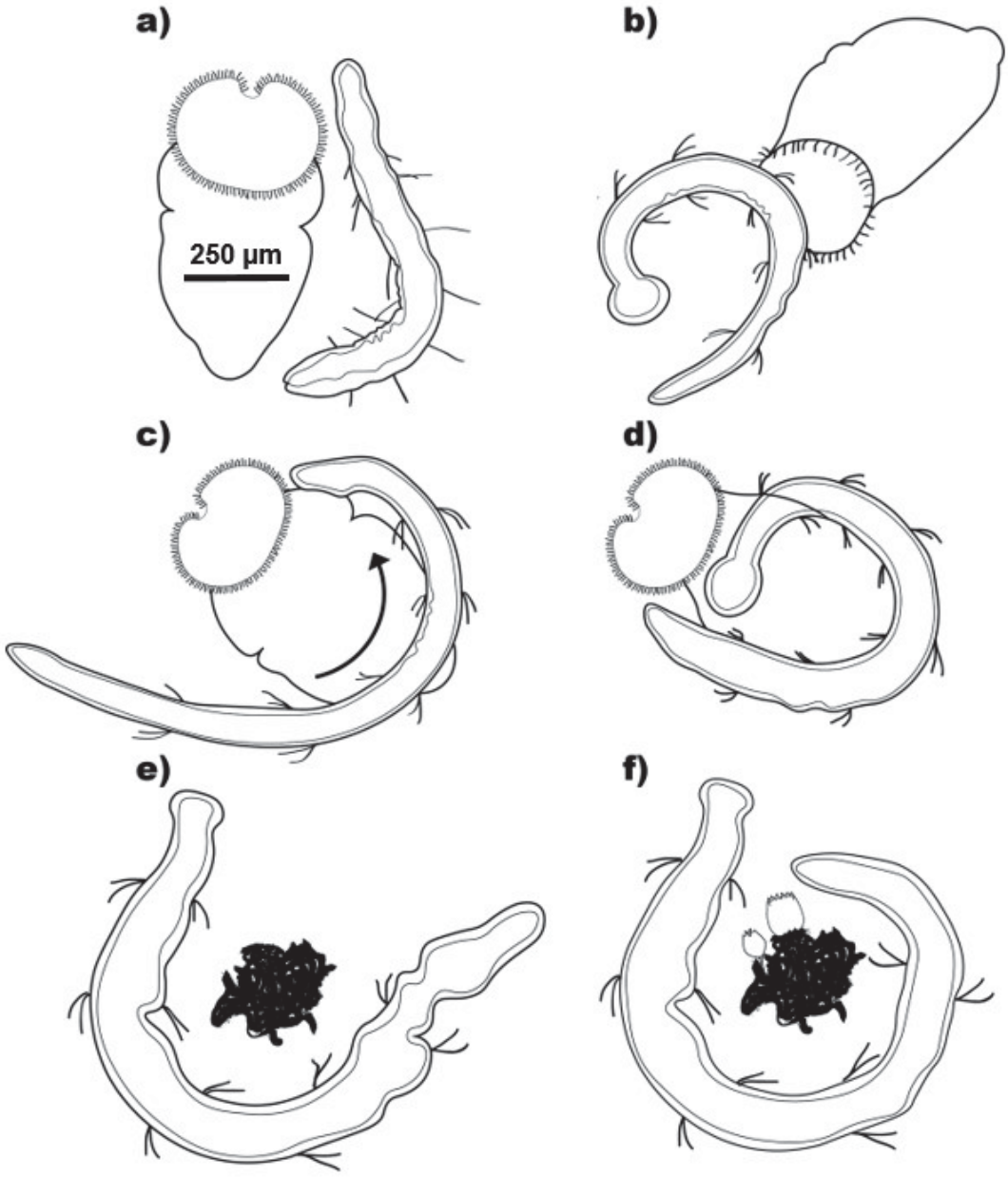

Figure 4. Feeding behavior of between oligochaete and rotifers species. 1) *Aeolosoma hemprichi* vs *Asplanchna brightwellii*, 2) *A*. *brightwellii* attempt to devorate to eat *A*. h*emprichi*, 3) *A*. *hemprichi* curls to avoid being eaten, 4) *A*. *hemprichi* hug, 5) *A*. *hemprichi* detritus pellets formation by curls and hug, 6) *B*. *calyciflorus* and *L*. *quadridentata* trap into detritus pellets of *A*. *hemprichi*.

produces detritus pellets.

Link to see the video:
https://youtu.be/X14s0_K5g7k.

Discussion

Oligochaetes feed on decaying inorganic matter they produced themselves and deposit as detritus pellets with mucilage in which, eventually, rotifers are trapped. Owing to the above, it is a scavenging species and so it makes the most of the resources. The presence of oligochaetes in the rotifers' culture medium causes a drastic decrease of *B. calyciflorus*, a planktonic species, unlike the benthonic species *L. quadridentata*. Probably because oligochaetes naturally interact more with benthonic species like *L. quadridentata* in aquatic ecosystems, and so they are not affected, as it is the case for *B. calyciflorus*. Species of genus *Lecane*, characteristically live in benthonic habitats, preferably in the lower sediment (shallow part of the littoral), where they feed on bacteria and detritus (Serranía-Soto & Sarma 2003). Because of this, they have been considered indicators of the quality of water as regards the amount of organic matter (Fialkowska *et al*. 2016). They have also been reported in ecosystems where vegetation and roots prevail (Wallace *et al*., 2006). Moreover, it is common to discovery living with *A. hemprichi* in residual water from treatment plants, sediments, and owing to its biological and ecological characteristics, it can be a good indicator of changes in the concentrations of heavy metals and xenobiotics present in aquatic environments and water treatment plants (Kuniyasu *et al*. 1997; Song & Chen 2009; Falconi *et al*. 2015).

The oligochaete decreases its population when it grows together with predator *A. brightwellii*. Over interaction it was documented how the rotifer tried to eat the oligochaete, but it did not manage to as the oligochaete is able to curl and avoid being eaten by the rotifer; moreover, the oligochaete has bristles over its body that prevent it from being eaten.

Studies carried out by Nandini & Sarma (2004) demonstrated that *A. hemprichi* is capable of decreasing the population of cladoceran species *Alona rectangula, Ceriodaphnia dubia, Daphnia pulex* and *Moina macrocopa,* however, for the species *Macrothrix triserialis* no population decrease is noticed. By contrast, it has been reported that in natural conditions rotifer *A. brightwellii* is capaeble of eating cladocerans such as *Moina micrura* (Rubio-Franchini & Rico-Martínez 2011). Which suggests that *A. hemprichi* is tertiary consumer, while *A. brigthwellii* is secondary consumer of primary consumers such as cladocerans and rotifers. The mechanisms and the strategy to reduce the population of rotifers and cladocerans are favored by the mucilaginous exudate and the formation of detritus pellets in which they will be trapped. Also, there are reports of the presence of cladoceran *Moina micrura* that speeds up the process of death in species *B. calyciflorus*, and the species *B. calyciflorus*, as a response to stress, produces more eggs but with poor

quality as hatching reduces; on the contrary, in the absence of cladoceran *M. micrura*, the rotifer lays eggs with better hatching quality (Aranguiz-Acuña *et al*., 2014). *A. hemprichi*, in this case, kills *B. calyciflorus*, it prevents the former from reproducing.

Nandini & Sarma (2004) mention that *A*. *hemprichi* eats the valves of cladocerans and does it with the mouth of the oligochaete, i.e., by means of suction, which is physically aided by the bristles as they scrap the tissues. After this, oligochaetes defecate the decaying organic matter. The process of formation of detritus pellets with circular movements and stretching the body, it curls and this way it compacts the detritus and produces the pellets. There, it feeds on all the decaying matter owing to its capacity to absorb nutrients from the environment. In reports by Jamieson (1981), it is mentioned that oligochaetes have a specialized epidermis through which they absorb nutrients and light-weight molecules.

Explaining interactions between species is complex. As exercise to simply or clarify this idea, for example, and due to the abovementioned: first, *M. micrura* affects *B. calyciflorus*, and *A. brightwellii* eats *M. micrura*. Second, for its part, *A. brigthwellii* induces spines in *B. calyciflous*. Third, *A. hemprichi* affects all this zooplankton species. *M. micrura* and *A. brigthwellii* and *B. calyciflorus*. The succession life cycles and niches will determine these species survival.

In rotifers, the genus *Asplanchna* is voracious predator, in fact they are omnivorous and detritivores, plus there are *Asplanchna* morphotypes and trophi forms specialized by significant influence from food choices and the environmental factors that have effects on predator-prey interactions (Chang *et al*. 2010). For example, in the literature reported, rotifers of the genus *Asplanchna* are capable of inducing spines in rotifers, as a defense mechanism before predation that has been observed in detail in genera *Brachionus* and *Keratella* (Sarma *et al*. 2011; Bertani *et al*. 2012). However, it was not observed spines formation in *Brachionus calyciflorus* when it grows with *Aeolosoma hemprichi*.

The feeding behavior of *A. hemprichi* is fascinating, its contribution as detritivores, as well other species, is essential for the trophic transfer energy in aquatic ecosystems. In laboratory conditions, for example, rotifers and oligochaete interactions it is an appropriate species to study the movement of pollutants in the biota, analyzing the bioaccumulation and biomagnification of xenobiotic. According to Pérez-Yañez et al., (2019), *A*. *hemprichi* is an excellent indicator of metal bioaccumulation, due to its detritive characteristics and because it is considered a tertiary consumer. Finally, the formation of detritus pellets is not exclusive to *A. hemprichi*, the organisms that make this are described as engineers of the ecosystem, which directly or indirectly modulate the availability of resources for other species (Jones *et al*. 1994).

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Abstracts of the 16th International Rotifer Symposium, 2022

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Rotifers of lake psammon: A knowledge synthesis

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Most information on rotifers living in lake sands comes from the 1930s, when the first reports on this subject by Jerzy Wiszniewski appeared. This discovery caused a short-term increase in interest in psammon among the biologists of the world, which resulted in the appearance of several studies devoted to this habitat in this and the next decade. The publications from this period are still an important source of information about the characteristic features of psammolittoral and its organisms, as in the following years there was a decline in the interest in lake psammon. The research on the ecology of the lake psammon has returned in the last decade of the XX century and the studies included a description of the species structure and functioning of psammon rotifers, as well as the influence of mechanical, physical and chemical factors on psammon organisms. The analysis of literature materials and the results of my own research shows that although we have enriched significantly our knowledge about the ecology of psammon communities of Rotifera since Wiszniewski's discovery, the species richness of this group in geographical terms is still unknown. Due to the small number of studies in this area, we also do not know the level of endemism among the psammon rotifers. The role of psammon rotifers in benthic food webs is another issue requiring research.

DNA repair and homologous recombination during non-reductional meiosis in the asexual rotifer *Adineta vaga* and the importance of horizontal gene acquisition

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Bdelloid rotifers are microscopic animals notorious for their long-term persistence in the apparent absence of canonical sexual reproduction. This evolutionary paradox is often counterbalanced by invoking their ability to repair high levels of genome breakage caused by desiccation and other genotoxic stresses. How bdelloid rotifers persist despite the expected negative consequences of asexuality and how they survive extreme conditions has been the focus of my research group. We employ population and comparative genomic analyses to study evolutionary processes and recently started to explore the molecular mechanisms of their hyper-resistance to desiccation and radiation. I will present the chromosome-scale genome assembly of our model system *Adineta vaga*, having homologous chromosomes

with signatures of recombination. Genomes from clonal lines maintained in the laboratory have been sequenced at different timepoints and I briefly present the genome changes in *A. vaga* and the prevalence of homologous recombination. By studying spatiotemporal dynamics of DNA damage response in *A. vaga*, we found that repair of germline genomic lesions is delayed to a specific time window of oogenesis during which homologous chromosomes adopt a meiotic- like juxtaposed configuration. *A. vaga* undergoes a non-canonical meiosis allowing homologous chromosomes to pair and recombine without segregating into haploid gametes, as in automictic parthenogens. It results in a faithful reconstitution of the genome in the offspring, while DNA repair in somatic nuclei occurs within 24h after recovery, producing a partially reassembled genome. Finally, some of the first molecular actors identified in *A. vaga* that might play a role in their extreme resistances are presented.

Rotifers in ecological studies of small, shallow and macrophyte-dominated aquatic ecosystems

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The constant global change of our natural environment underlies the urgent need to understand but also to assess the speed and direction of changes. Therefore, serviceable monitoring tools must be developed and the application of biological communities can serve this purpose; particularly methods that can assess both biodiversity and diagnose water quality conditions. Rotifers provide suitable community indices to identify the response to abiotic and biotic changes, and habitat type in reference to the level of human stress in the catchment area. Macrophytes provide rotifers with diverse microhabitats, whose complexity primarily determines the structure of species and functional groups distribution. In the case of small and shallow ecosystems aquatic vegetation plays a particularly crucial role. However, these ecosystems have not been included in ecological studies for years but are immensely important in water retention but especially in preserving regional and global diversity. The presence of macrophytes, particularly of elodeids, increases overall diversity, the occurrence of valuable species for maintaining high ecological status and share of littoral rotifers, but it also contributes to the reduction of rotifers characteristic of eutrophic conditions. The degree of human transformation governs the occurrence of specific rotifer communities but despite strong anthropopression (e.g. urbanisation or farmland) macrophyte-dominated water bodies still remain a source of increased diversity including rotifers. All the above certify the considerable suitability of rotifers for environmental studies, as they swiftly and reliably respond to changes in their environment.

The undiscovered country: grand challenges in rotifer biology

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Various authors have attributed the statement "All science is either physics or stamp collecting" to the Physicist, Ernest Rutherford. Putting this sarcastic quip aside, we know that scientific disciplines come of age only when they can generate testable, repeatable, and falsifiable hypotheses; yet each discipline begins by simply collecting observational information. It is clear, even with a casual assessment of the previous 15 International Rotifer Symposia (IRS) and the extensive literature published since our first congress, that rotifer research has moved well beyond merely describing species, making lists of their occurrences, and describing changes in their population dynamics. While our discipline has come of age, and in spite of its excellent progress, we believe more remains to be done. Here we nominate several grand challenges in rotifer research; accordingly, we need to advance our understanding of rotiferan (1) neurobiological connectomes, (2) genomic architectures and control systems, (3) cellular physiology, (4) life histories in light of their acanthocephalan relatives, (5) ecological responses to stresses, (6) biogeography and distribution of cryptic species, (7) morphospace (trophi, jointed appendages, and body form), and (8) evolution within the context of all gnathiferans. Additionally, researchers must give preference to model species other than *Brachionus* spp., the white mice for rotiferologists. Besides these fields of study, rotiferologists should (9) re-establish educational venues, either in a convenient physical place and/or electronically and (10) make reliable, internet-based databases widely available to all. We expect contributions to IRS-16 will provide new information in these and other fields.

Acanthocephalans, fish intestinal parasites, as bioindicators of water quality in the freshwater ecosystem

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Acanthocephala is a phylum of endoparasites hosted by Mandibulata and Gnathostomata, and phylogenetically related to Rotifera. Acanthocephalans

lack digestive system, and therefore depend on host micronutrients, which are absorbed from the fish intestine. This especially involves essential metals, as elements of physiological importance, but also toxic elements which are absorbed even more effectively in acanthocephalans than in other commonly used bioindicator organisms, like fish, crustaceans, and bivalves. Thus, novel field Environmental Parasitology investigates linkage between contamination and parasitism, and possible application of acanthocephalans as indicators in environmental risk assessment studies. Estimation of metal exposure in Croatia was conducted in the lowland Sava River, using *Pomhorhynchus laevis* and *Acanthocephalus anguillae* hosted by European chub (*Squalius cephalus*) and in the karst Krka River, using *Dentitruncus truttae* hosted by brown trout (*Salmo trutta*). Acanthocephalan prevalence was much higher in fish from Krka than the Sava River and all acanthocephalan species showed higher metal accumulation than intestinal tissue of fish host. Bioconcentration factor (BCF), the ratio of the element concentration in the parasites to that in host gastrointestinal tissue (BCF = C[parasite]/C[host intestine]), confirmed especially effective accumulation of toxic elements in acanthocephalans, like Ag, Cd, and Pb. Presented research confirmed acanthocephalans as valuable biological indicators of the water quality, which should be combined with a set of physical, chemical and biological parameters, and might in the future be correlated with dynamics and diversity of the free-living rotifers in the freshwater ecosystem for the purpose of comprehensive estimation of environmental health.

Bet hedging in rotifers: optimal diapause strategies in variable environments

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Bet hedging (BH) is hypothesized as an adaptation evolving in unpredictable environments. It is defined as a strategy that reduces across-generation variance in fitness at the cost of reducing arithmetic mean fitness either through the production of a variety of phenotypes in a genotype's offspring (diversified BH) or a unique low-risk phenotype (conservative BH). While the empirical testing of BH has been rather elusive, rotifers and their habitats have been postulated as an appropriate study system for such assessment. Here we review our own results on BH in rotifer diapause traits, but also point out new lines of research currently underway. We exploit BH theory to propose accurate predictions on two key traits: the timing of diapausing egg production and their hatching fraction. Specifically, we expect that the higher the unpredictability in the length of the growing season of rotifer habitats, (1) the earlier the timing for diapausing egg production, and (2) the lower the hatching fraction. We provide correlational evidence supporting these predictions from field populations of

Brachionus plicatilis covering a gradient of environmental unpredictability. Further evidence is gained through the experimental study of evolving populations under predictable versus unpredictable divergent selective regimes. While our findings demonstrate empirically the existence of BH regarding unpredictability across growing seasons, diapause traits may still be shaped by other sources of unpredictability, such as that operating within a growing season whose onset is not reliable (false starts). This is a current research topic for which new predictions are being formulated and tested.

Exploring the mechanisms of Schistosome Paralysis Factor production in *Rotaria rotatoria*

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Schistosomes are parasitic flatworms that infect over 200 million people, causing the neglected tropical disease schistosomiasis. A single drug, praziquantel, is currently used to treat schistosome infection. The potential emergence of praziquantel-resistant strains makes developing strategies to prevent infection an important goal. Rotifers colonizing the shell of the schistosome's intermediate host snail (*Biomphalaria glabrata*) produce a water-soluble factor that paralyzes cercariae, the life-cycle stage infecting humans. Our subsequent results revealed that of the two rotifer species living commensally on the snail host, only *Rotaria rotatoria* produces Schistosome Paralysis Factor (SPF), a novel tetracyclic alkaloid that paralyzes cercariae and prevents infection of a mammalian host. To understand how *R. rotatoria* synthesizes this unique molecule we have begun developing genomic tools for studying this fascinating organism. Here, we present an update on our efforts to mine transcriptomic and genomic data for candidate alkaloid-synthetic genes and to develop functional tools for testing their requirement in SPF production.

Quantification of spatial dispersal and characterization of evolutionary traits associated with successful colonization in rotifers

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Dispersal is an essential component in the life history of any organism that has strong ecological implications. In this contribution we study the factors associated to dispersal dynamics and test quantitatively if specific mechanisms are at work. For this purpose, we use a well-characterised rotifer system, consisting of

three congeneric species (*Brachionus plicatilis, Brachionus ibericus* and *Brachionus rotundiformis*) that inhabit a Spanish Mediterranean coastal pond. For these species, we quantify short-distance wind-dispersal of diapausing eggs and study dispersal patterns in relation to the wind regime (direction and intensity), source pond status (surface of sediment exposed to the wind) and the abundance of diapausing eggs in the source populations of the three species. A trade-off between the two alternative fates of a diapausing egg (to disperse or not to disperse) exists, and a proportion of dispersing eggs is expected to respond to differential selective pressures and strategies for each species. The evolved mechanisms might involve polymorphism in the diapausing eggs, promoting one fate or another, and in the diapausing egg genotypes, being more or less oriented towards successful colonization. It would be expected that high investment in sexual reproduction would facilitate adaptation to new environments. In this regard, we study whether *B. plicatilis* dispersing genotypes constitute a positively biased sample from the genotype pool of the source population in relation to the propensity to initiate sexual reproduction and two morphological features (body and lipid deposit size) of the diapausing eggs putatively promoting successful dispersal.

Speciation genomics of bdelloid rotifers in the genus *Rotaria*

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Speciation if often thought to be synonymous with the evolution of reproductive barriers. Genome-wide divergence is only possible with restricted interbreeding and gene flow between species. Recent work has shown, however, that ecological divergence can occur even in the face of gene flow. In these 'speciation with gene flow' models, species diverge only at specific genome regions involved directly in the niche differences of the two species. Genome sequencing therefore provides a way to test the relative importance of selection and barriers to gene flow for speciation. Here, we apply this approach to bdelloid rotifers of the genus *Rotaria*. Unlike most eukaryotes, males and sexual reproduction have never been observed in the entire class Bdelloidea, making them an interesting test case for theories of speciation. We focus on two species, *R. socialis* and *R. magnacalcarata*, that live in close proximity on subtly different parts of the body of the water louse Asellus aquaticus. Using whole-genome resequencing of multiple isolates of both species, we test alternative models for their divergence based on theory for clonal divergence and possible alternative modes of gene exchange.

The molecular evolution of 18S rRNA in Rotifera and its phylogenetic implications

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Even in the postgenomic era, the gene for 18S rRNA remains a workhorse for elucidating higher-level phylogenetic relationships among animals due to it being widely sequenced, but also because the variable rates of evolution across the gene make it informative at numerous phylogenetic levels. Using both newly obtained and GenBank sequences, I mapped an alignment of 192 rotifer 18S rRNA sequences (162 monogonont, 35 bdelloid, and 1 seisonid sequence) against the eukaryotic core structure for 18S rRNA established by the European Ribosomal Database and derived the equivalent maps for three exemplar species (*Adineta vaga, Brachionus plicatilis* and *Seison nebaliae*) representing the major rotifer clades. All three maps showed a good fit to the eukaryotic core structure. A variably expressed deletion was present among bdelloids at the end of the V3 hypervariable region that extended into important structural regions of the molecule in some species. Inferred relative rates of evolution across rotifers were highly variable and followed the expected pattern of hypervariable regions being more variable than loops which, in turn, were more variable than stems. Up to 60% of the sites were constant. Large differences in the primary sequences between the three rotifer clades (unpaired average P-distances of ca. 20%) contrasted with highly reduced variability within each of Bdelloidea and Monogononta (ca. 2%) to yield three highly divergent, yet conserved motifs within rotifers. This finding, with its potential for creating long-branch attraction and other analytical artefacts might explain why higher-level relationships within rotifers remain unresolved to this day.

Kleiber's law and allometric scaling in colonial rotifers

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The repeated occurrence of coloniality across many disparate taxa suggests that a colonial lifestyle confers an adaptive advantage relative to a solitary lifestyle. For rotifers, one possible explanation is that colonies may provide an energetic advantage to their members. An energetic advantage may take the form of lower respiration rates for colony members relative to solitary animals. Respiration rate scales allometrically with size according to Klieber's law. For many colonial animals, colony respiration scales with colony size in the same manner that respiration rate scales with body size in solitary animals. To test whether coloniality provides an energetic advantage, we compared the allometric relationships of respiration rate and size in rotifer colonies (*Sinantherina socialis* and *Lacinularia flosculosa*) and non-colonial rotifers (*Hexarthra* sp. and *Euchlanis kingii*). Oxygen consumption was measured using a Loligo microplate system. Rotifers were measured and individual/colony volumes were estimated using ImageJ. The maximum colony size for *S. socialis* and *L. flosculosa* was 142 and 456 individuals, respectively. From colony volume and respiration, allometric

scaling factors were estimated through regression analysis. Colonies respired at ~1.3 pm/ind/min whereas solitary rotifers had higher respiration rates (e.g., 2.3 pm/ind/min in *E. kingii*). We estimated a scaling factor of ~1 for colonial rotifers, similar to scaling factors found for groups of individual animals. For example, we found scaling factors of ~1.2 and ~1 for *Hexarthra* sp. and *E. kingii*, respectively. These findings suggest that rotifer colonies may not confer an energetic advantage to their members.

The effect of different type of standard diet on the strength of phenotypic plastic body size response to temperature in the rotifer *Lecane inermis*

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Temperature is the most important parameter shaping life on Earth. This environmental factor has considerable impact on organisms, especially ectotherms. The Temperature-Size Rule (TSR) explains how temperature affects body size in ectothermal organisms, which achieve smaller size at warmer temperature and larger size at colder temperature. This rule was found to hold between minimal and optimal temperatures for population growth rate, a direct fitness measure. These organism-specific temperatures indicate thresholds between which organisms realize the plastic body size adjustment, while beyond this thermal range phenotypic plasticity is limited and body size is canalized. We examined the TSR response in the rotifer *Lecane inermis* under three different types of standard diet to find out whether food conditions affect the strength of TSR response within the optimal thermal range, and body size canalization beyond this range. We used temperatures which were selected as minimum and optimum for population growth rate in a previous study. Our results indicate that the type of diet affects either the strength of plastic body size response within the optimal thermal range, or the body size which is canalized beyond this range. These results show that even standard nutritional conditions may affect the results in the studies focusing on phenotypic plasticity and shed light on patterns associated with optimal resources allocation in the context of TSR.

Long-term tracking of the invasion process of *Kellicottia bostoniensis* in a tropical reservoir

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Kellicottia bostoniensis (Rousselet, 1908), native to North America, was first introduced to South America in 1990, probably via ballast waters. The present framework aimed at evaluate a dynamic, temporal component of its invasion process. We analysed abundance and functional aspects of the recipient community and of *K. bostoniensis* using data from a nine-years monthly monitored reservoir in Brazil. *K. bostoniensis* was present since 2001, in 20% of the 566 samples. Higher abundances occurred during the mixing of the water column during winters, when it accounted for up to 60% of the total rotifer assemblage. Multiple regression analyses revealed abundances of *K. bostoniensis* negatively correlated with water temperature and positively with the epilimnion depth, indicating a possible environmental trigger from sleeper populations. *K. bostoniensis* and other 66 rotifers were considered as small microphages (SM), 16 as large microphages (LM) and 28 as raptorial (RP). From 2001 to 2003, RP were correlated with all SM (+0.68), from 2004 thereafter RP were mostly correlated with SM without *K. bostoniensis* (+0.46) than with this invader (+0.21), and from 2007 to 2009 abundances of *K. bostoniensis* correlated with LM (+0.16). Mean body length of *K. bostoniensis* increased from 2001 to 2009, showing a tendency to be included among the LM (>250um). Fast life-history strategies and morphological and functional plasticity may allow rapid population recruitment over large-scale temporal dynamics, influencing invasion success. We also advocate that species traits, ecosystem types, or community contexts must not be neglected to codetermine invasiveness.

Transgenerational effects on sexual reproduction in *Brachionus plicatilis* populations in relation to the environmental predictability of their habitats

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Understanding the processes that enable adaptation of organisms to time-varying environments is critically relevant in evolutionary ecology. A way to cope with environmental fluctuations where predictable conditions affect several generations of individuals is through non-genetic transgenerational effects. The phenotype of ancestors affects the phenotype of their descendants matching it with the expected environment of the latter. Facultatively sexual rotifers inhabiting water bodies that cover a wide gradient of environmental predictability in Eastern Spain are a good study model for this topic. In their life cycle sex is linked to diapausing-egg production that enables survival between growing seasons. In several rotifer species, sexual reproduction is inhibited in several generations after diapausing-egg hatching. We hypothesized that in ponds where the growing season length is more predictable, rotifer clones proliferate asexually longer, hence allowing a fuller exploitation of the growing season and therefore maximize diapausing-egg production by the end of the season. We tested this prediction by estimating the proportion of sexual females

produced by eight clones of the rotifer *Brachionus plicatilis* inhabiting eight ponds (8x8= 64 clones) from our study system. Analysis based on GLMM showed that rotifer clones from more predictable ponds were unresponsive to sex-inducing cues for a higher number of generations after leaving diapause. However, clones for more unpredictable ponds did respond from early generations likely as a way to ensure the production of diapausing eggs against an unexpected end of the growing season. Significant clonal variation was found in the transgenerational response at the population level.

Population genomics and adaptive divergence analysis in rotifers from deep tropical lakes

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Organisms that inhabit endorheic lakes in semi-arid regions face contrasting environmental factors that result in a mosaic of selection pressures. Therefore, identifying the genetic bases of adaptation to these environments with insular distribution is a challenge to understanding how biological diversification occurs. We studied three populations of rotifers belonging to the *Brachionus plicatilis* complex (B. sp. 'Mexico'), in which it has been described adaptation to local conditions of salinity despite they inhabit three deep crater lakes closely located in Oriental Basin, Mexico. These lakes are limnologically different, two are saline and one is freshwater. We obtain a reduced representation of the genome using sequence-based genotyping (SBG) for single nucleotide polymorphisms (SNPs) to analyze the genome-wide differentiation and population structure. We identified and genotyped 1790 high-quality SNPs, 137 of them were found to be putatively under selection with signatures of diversifying selection. Populations are genetically differentiated showing a pattern of population structure and constitute three monophyletic lineages one from each lake. Using the published draft genome for *B. plicatilis*, putative functions were associated with some of the loci under selection, however, they showed a variety of genetic ontologies, making it difficult to obtain reliable inferences. Our results showed genomic evidence of adaptation to local conditions fueled by standing genetic variation, which has promoted the reduction of gene flow and fostered the accumulation of genomic differentiation in rotifer populations.

Ecological stoichiometry of rotifers

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In many aquatic food webs, rotifers play an important role linking primary producers and secondary consumers and

as recyclers of biogenic elements. According to ecological stoichiometry, trophic interactions and nutrient cycling are strongly affected by stoichiometric mismatch (i.e. the difference in elemental composition between food and their consumers). Stoichiometric mismatch has profound impacts on the growth performance of zooplankton herbivores, their grazing pressure on phytoplankton and the relative rates at which they recycle nutrients. Compared to large zooplankton (*Daphnia*), the ecological stoichiometry of rotifers has remained poorly explored. After a short introduction of ecological stoichiometry as a research discipline the work that has been done in this field on rotifers will be explored. Evidence will be provided on the strong negative impacts of stoichiometric mismatch between rotifers and their food resources, caused by both nutrient limitation and nutrient excess. It will be shown that phosphorus limitation of phytoplankton affects rotifer growth and life history not only directly (i.e. through elemental limitation) but also indirectly through alterations in other quality related phytoplankton features (e.g. morphology, fatty acid content). The strength of rotifer elemental homeostasis will be explored along a broad gradient of food carbon to phosphorus ratios, and the potential ecological implications of its non-linear response will be discussed. Finally, the evolutionary potential of rotifer populations to adapt to elemental mismatch and its eco-evolutionary implications will be considered. Current knowledge gaps will be discussed and an outline of promising research directions provided.

Fire and water: Effect of forest fires on soil rotifers from a long-time perspective

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Rotifer communities are very common and abundant in soils that frequently dry out. However, being part of the hydrobiont microfauna, they are well adapted to desiccation. Dry forests on sandstone rocks are susceptible to forest fires, which are relatively frequent in the area of the Bohemian Switzerland National Park in the Czech Republic. We studied the effects of such disturbance on various groups of soil fauna on a chronosequence of burned areas ranging from recent to more than a hundred years old. Abundance of rotifers varied between 104 and 106 individuals m^{-2}. While bdelloids dominate the soils, monogononts are also regularly present. The results suggest that although the rotifer community is severely impacted after fire, it can recover within a short period of time and that the age of the plot has minimal effect on rotifers in the long term. On the other hand, the effect of fire severity is highly significant. Among others, *Wierzejskiella vagneri* strongly avoids burned plots more than unburned ones. We can say that a severe fire strongly changes the conditions in the soil profile by removing mainly the upper litter layer, which in turn affects the rotifer community.

Highly efficient CRISPR-mediated gene knockouts reveal biological function in *Brachionus manjavacas*

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There is an increasingly extensive genetic tool kit available for monogonont rotifers of the *Brachionus* species group, including RNAi protocols and multiple genomes and transcriptomes. Here, it is reported a protocol for highly efficient CRISPR-mediated mutations in *B. manjavacas* though microinjection of Cas9 protein and synthetic single guide RNA (sgRNA) into the vitellaria of young amictic females. Females were injected with sgRNA to either the developmental gene vasa or the DNA mismatch repair gene mlh3. Sequencing of offspring (F0) produced from injected mothers reveals most carry at least one CRISPR-induced mutation, with many apparently mutated at both alleles or mosaic. Observed mutations include short- to mid-size deletions (2-18bp), mid-size insertions (6-14bp), and 1-2 bp replacements, indicating a variety of repair mechanisms following Cas9 endonuclease activity. F0 individuals with mutations in vasa appeared normal; however stable clonal lineages with mutant alleles could not be established, suggesting that vasa is a maternal effect gene required for development. In contrast, F0 individuals with mutations in mlh3 displayed a variety of alterations in morphology and fecundity, suggesting that this gene plays a critical but non- essential role in the amictic germ line. Clonal lineages have been established from F0 mlh3 mutant individuals that are heterozygous with wildtype, homozygous for single mutation types, or contain two different mutation types. These results demonstrate the efficacy of the CRISPR- Cas9 system in rotifers and how CRISPR-mediated gene knockouts can provide insight into the function of specific genes to further advance rotifers as a model system for biological discovery.

Integrated approach for cyanobacteria removal: grazing by rotifers and application of aeration and hydrogen peroxide

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Cyanobacterial blooms are increasingly becoming a serious problem all over the world. The genus *Microcystis* is among the most frequently encountered cyanobacterial genera in freshwater ecosystems. Its characteristics include colony formation, large size, buoyancy in the water column and production of secondary metabolites. Blooms of *Microcystis* pose a risk to the health of

aquatic communities and humans. Biological strategies focused on the use of crustacean zooplankton are successful in controlling cyanobacterial blooms, mostly in temperate regions. In eutrophic aquatic systems in Mexico, rotifers are the dominant component of zooplankton with cladocerans being infrequent. The use of physical and chemical strategies has shown contrasting results. In this work, the combined effects of aeration, hydrogen peroxide levels (0.5 and 1.0 mgL^{-1}) and grazing by the rotifer *Plationus patulus* to control blooms of *Microcystis* spp. were evaluated. Aeration influenced the disintegration of larger colonies (>250 µm) leading to an increase in the number of single cells. Hydrogen peroxide at concentrations greater than 1.0 mg L^{-1} affected the survival of *P. patulus*, but still caused significant changes in the abundance in cyanobacterial colonies. Grazing by *P. patulus* decreased the number of unicellular cyanobacteria by about 50%. Our results suggest that the application of combined of physical (aeration), chemical (peroxide) and biological (grazing by rotifers) factors was more effective than any one of them alone in controlling cyanobacterial blooms in eutrophic waterbodies.

Comparative phylogenetics to elucidate the evolutionary effects of parthenogenetic reproduction in rotifers

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Bdelloid rotifers have been notorious among evolutionary biologists for their absence of males and sexual reproduction, so much as to be named an evolutionary scandal. Recent studies started questioning such obligate asexuality, with genomic evidence of recombination. It is thus still unclear whether bdelloids are completely asexual or not and the evolutionary effects of lack of sex or rare sex in bdelloids have not yet been assessed with a phylogenetic approach. Our aim is to compare phylogenies from a mitochondrial marker (COI) and a nuclear marker (ITS1) across many individuals of few selected species in bdelloids and in monogononts. The two markers should move across individuals and populations due to gene flow and evolve independently in monogononts, whereas in bdelloids they should be linked in the same genomic history within each clonal lineage if no recombination occurs. A stronger phylogenetic congruence between the two markers in bdelloids than in monogononts will support the idea of bdelloids diversifying in the absence of males or sexual recombination.

Traits of species in genus *Ptygura* with the antler-like cervical hooks point to a taxonomic relocation of the sessile rotifers

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After the recent description of the rare rotifer *Ptygura innominata* Franch, 2021 (Mallorca, Spain), observation of new specimens offers supplementary traits of its life cycle, morphology, and ethology.

I describe the not previously observed eggs, free swimming larva, the foot and adhesive disc of adult females along with a more detailed description of the dorsal plate and trophi after bleach treatment. I compare *Ptygura* species bearing antler-like cervical hooks with other species of *Ptygura* genus, not bearing this kind of hooks, found in the sample site (*P. ctenoida*, *P. longicornis* and *P. stygis*), as well as with other *Ptygura* species and Flosculariidae taxa. Differences in trophi morphology and function of lipid droplets are discussed. The similarities between the dorsal plates of *P. ctenoida* and *P. innominata* provide evidence of a close relationship between all the *Ptygura* species having stiffened anatomic elements. *P. innominata* shows a rake-like movement of cervical hooks to remove floc masses and eventually incorporate them into the tube construction, as previously described only for some *Limnias* species. The corona of *Ptygura* species bearing antler-like cervical hooks is incomplete at its base and oriented according to the frontal plane, making this corona layout unique in the genus. The findings support the previously proposed hypothesis that the *Ptygura* species bearing stiffened dorsal plates are phylogenetically closer to *Limnias* and *Beauchampia* than to the rest of *Ptygura* species.

Sequential anterior expression domains of an ancient *Fox* gene cluster in *Brachionus manjavacas* indicate roles in body plan regionalization

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Fox genes encode a group of evolutionarily old transcription factors dating back at least to the last common ancestor of metazoans and fungi. Based on similarities of signatures within the forkhead domain characterising these proteins, this gene-rich group has been divided into 24 protein families (FoxA to FoxS). Fox transcription factors play important roles not only in developmental processes but also in cell cycle regulation and metabolism. Across the animal kingdom some developmental regulatory gene classes, such as *Hox*, *ParaHox* and *NKx* genes, are often arranged in evolutionary conserved genomic clusters. In gnathiferans studied so far however, these clusters are dispersed. Genomic linkage has been shown for three *Fox* orthologs, *FoxL1*, *FoxC* and *FoxF*, in Ecdysozoa, Lophotrochozoa and Deuterostomia. Expression of these genes is often associated with mesoderm development. Focusing on characteristics of the *Fox* gene complement of Gnathifera first, *Fox* orthologs were isolated from genomic data of *Brachionus manjavacas* (Monogononta), *Adineta vaga* (Bdelloidea), *Seison nebaliae* (Seisonida) and *Pomphorhynchus laevis* (Acanthocephala) and assigned to their respective families by phylogenetic

analysis. In general, the gnathiferan *Fox* complements exhibit higher similarity to ecdysozoan than lophotrochozoan complements. Intriguingly, mapping of the sequences to genomic scaffolds revealed the ancient *Fox* cluster to be intact in *B. manjavacas*. Second, analyses of expression of *Bm-FoxL1*, *Bm-FoxC* and *Bm- FoxF* in developmental stages showed their sequential activation in anterior expression domains during morphogenesis. Collectively, these results support co-option of *FoxL1*, *FoxC* and *FoxF* genes for patterning of the anterior body plan in Gnathifera, indicating a possible functional coupling.

World of rotifers on blockchain

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The idea of open data has been highlighted as very important for the advancement of research, especially in environmental science, climate change studies, and biology. However, there are still many concerns about publishing data as open data, such as loss of credit. While there are many data archives and repositories on the Internet, data are mostly scattered, and the use of technical innovations in data sharing still needs improvement. We propose a customised blockchain solution based on both permissioned and permissionless blockchain implementations to acquire, match, and harmonise harmonise Rotifer datasets in an immutable open data format for secure and trusted access and analysis. The proposed system supports intuitive metadata constructs for Rotifer datasets bound to a predefined model that provides flexibility and consistency for data consumers. The goal is to create a decentralised system for voluntary collection of rotifer metadata with a standardised licence for attribution and authorship that allows data users, data providers, and data managers to easily access and reuse the data. The approach can also be applied to other biology and environmental data, which can foster AI development by incorporating metadata into the blockchain so that it can detect patterns, assess changes, or suggest actions to restore the object under study.

Differential effects of the consumption of different gold nanoparticles on the longevity and reproduction of the planktonic rotifer *Brachionus calyciflorus*

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Gold nanoparticles (AuNPs) are widely used in diverse fields including catalytic chemistry, biomedicine, or electronics and can enter the aquatic environment through sewage and abrasion to act as particle pollution for limnic microorganisms like

rotifers. Through different ligands such as citrate (Cit), polyvinylpyrrolidone (PVP), or cetyl-trimethyl-ammonium-chloride (CTAC), AuNPs are tailor-made for different applications, resulting in particles with different physical and chemical properties. However, the effects of different AuNPs after their ingestion by microorganisms are largely unexplored. Therefore, we exposed the limnic rotifer *Brachionus calyciflorus* to AuNPs with ligands of either Cit, PVP, or CTAC. Ultrastructural analyses revealed the ingestion of all three AuNP types with further internalization in the stomach cells. However, different significant effects were observed in the resultant mortality and reproductive rates. Compared to control animals, individuals exposed to AuCit and AuCTAC NPs showed significant decreases in both longevity (68.5 ± 3.5 h and 2.5 ± 0.2 h, respectively; mean ± SEM, both n = 48) and number of offspring per individual (2.1 ± 0.3 and 0.0 ± 0.0; mean ± SEM, both n = 48) (control = 97.0 ± 6.0 h and 4.8 ± 0.6 offspring). By contrast, individuals exposed to AuPVP NPs showed no change in longevity (98.8 ± 5.5 h) but produced significantly more offspring per individual (7.0 ± 0.9; mean ± SEM, n = 47). Our findings therefore reinforce that different types of AuNPs can have different impacts, even on the same organism, thereby making a general assessment of their environmental impact highly complex.

Ecological and genetic studies of rotifers from littoral zone in Presa del Llano reservoir, Villa del Carbon, Mexico

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Rotifers are a polymorphic group with worldwide distribution. Being the trophic link in waterbodies where they feed on phytoplankton and detritus, they transfer energy from lower to higher trophic levels. They are also sensitive to changes in the environment. Water temperature, pH, salinity and food concentration are some of the factors that control their diversity and abundance. In the last two decades the occurrence of many endemic rotifer taxa has been reported, limiting the distribution of some species to certain geographic areas. More than 400 species of Rotifera are known from Mexico, but this number could be an underestimate as the presence of cryptic speciation has been reported in several genera of rotifers. In this study we present data on the seasonal changes (Aug. 2021 to July 2022) in the rotifer species richness and abundance from a high altitude (2850 m.a.s.l.) waterbody, Llano reservoir (State of Mexico, Mexico). We also analysed sympatric populations of some common monogonont rotifer species using mitochondrial (COI) and nuclear (ITS) markers. Total rotifer density ranged between <350 and >2300 ind. L^{-1}, while the diversity indices were between 2.1 and 3.3 bits ind^{-1}. Selected species from the dominant genera such as *Keratella*, *Trichotria*, *Trichocerca*, *Mytilina* and *Lecane* were considered in this study.

Evolution barrier in two morphotypes of the freshwater rotifer *Brachionus angularis*

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In Central Mexico different morphotypes of the *Brachionus* genus have been detected in *B. angularis, B. bidentatus, B. quadridentatus*, and *B. calyciforus*. Some of the morphotypes are considered as cryptic species. However, to determine species status of the morphotypes integrative studies that combine solid taxonomic features and confirmation by DNA analyses are required to avoid misunderstandings and preclude correct species classifications. In this work, an integrative examination was made to differentiate two morphotypes of the freshwater rotifer *Brachionus angularis* that coexist in the same water pond. We employed a) DNA barcoding with ITS and COI genes, b) differentiation of taxonomical features in diapausing eggs, females, and males, and c) sexual reproductive behaviour (counting the number of rods and spermatozoa, resting eggs production, and cross-mating behaviour) to determine evolution barriers between the two morphotypes. Preliminary results demonstrate strong evidence of divergence between the morphotypes in all aspects suggesting evolutionary mechanisms such as size of the organism (female and male), ecological niche determined by physical and chemical parameters, and sexual barriers (number of spermatozoa) may play a fundamental role in speciation. We suggest that different environmental conditions and not the hybridization force these two morphotypes to diverge.

Comparative sexual reproductive sensitivity in the marine rotifer *Brachionus plicatilis* and *Brachionus rotundiformis*: A link with stress adaptations

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Environmental factors such as temperature affect the bioavailability or toxicity of substances (i.e., trace metals, ammonia), while habitat-specific adaptations may regulate the reproductive stress responses of organisms. Two marine rotifers *Brachionus plicatilis* and *Brachionus rotundiformis* originating from temperate, respectively tropical waters, have been subjected to differing habitat-resource stress during their evolutionary history and may have evolved specific coping strategies regarding future threats. Here, we compare the tolerance and reproductive adaptations of the two rotifers upon exposure to the trace elements iron ($FeSO_4 \cdot 7H_2O$, 0–96 mg/L) and zinc ($ZnCl_2$, 0–2 mg/L), as well as to unionized ammonia (NH_3-N, 0–29.3 mg/L). The two rotifers exhibited species-specific responses in terms of sexual reproductive sensitivity, lipid metabolism (citrate lyase, mitochondrial cytochrome), and anti-stress defences (CuZnSOD, cat, CYP clan

2&3). Compared to asexual reproduction, sexual reproduction of *B. plicatilis* was more susceptible to Fe, Zn, and NH3-N stress, which was supported by significantly inhibited resting egg production. However, in *B. rotundiformis*, the same treatment resulted in enhanced resting egg formation. These effects were associated with different responses regarding neutral lipid contents in reproductive organs and oxidative/antioxidant metabolisms between the two species: lipid contents and defence activities were inhibited in *B. plicatilis*, whereas the opposite phenomenon was observed in *B. rotundiformis*. These results highlight the importance of lipids in maintaining rotifer sexual reproduction. Furthermore, due to the tropical habitat experience, rotifer *B. rotundiformis* may have evolved greater reproductive flexibility to upcoming stress.

Adapting to an increasingly stressful environment: benefits of micro-evolutionary adaptation in rotifers are non-linear.

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In a changing world, organisms may be able to adapt to gradually increasing stress levels through rapid micro-evolutionary adaptation. However, stress levels tend to fluctuate with time and long-term increases in average stress levels are often associated with increasing amplitudes of stress levels. Hence, the occurrence of peak stress levels, e.g. during the occurrence of extreme events, may eventually still result in the extirpation of populations unless adaptation to low stress increases the coping abilities of organisms to deal with the influence of higher stress levels. To test this idea, an evolution experiment was conducted allowing replicate, genetically identical populations of the freshwater monogonont rotifer *Brachionus calyciflorus* s.s. to adapt to four levels of copper stress (0, 45, 57.5, and 62.5 µg/L). All populations were then exposed to each of three copper treatments (45, 57.5, and 62.5 µg/L). Treatments with the highest copper concentrations (57.5 and 62.5 µg/L) strongly reduced population growth rates of control populations, but much less in copper-adapted populations. At low copper concentrations (45 µg/L), the performance of control populations showed no tendency to be higher than that of adapted populations, indicating the absence of a trade-off. Interestingly, populations with a 45 µg/L copper selection history demonstrated a much higher ability to cope with much high copper concentrations (57.5 and 62.5 µg/L) than the control populations. Results show that evolved tolerance to low levels of a stressor may enhance the ability to cope with much higher levels of the same stressor.

Evidence of functions contributing to desiccation resistance and resilience from analysis of transcription patterns in *Adineta vaga*

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Desiccation-tolerant bdelloid rotifers such as *Adineta vaga* can enter and recover from anhydrobiosis at any life stage with no loss of lifespan or fecundity. Such bdelloids inhabit ephemeral waters, including water films where conditions may be unstable and unpredictable, implying that they have evolved under strong selection for resistance and resilience to environmental stress. To identify potential functions related to desiccation survival we compared transcript levels in *A. vaga* under conditions of stable hydration, entry into, and recovery from anhydrobiosis. About 25% and 35% of genes had a significant change in transcript levels upon entry or recovery, respectively, while about 20% maintained significantly equal levels in all three conditions. There were few significantly enriched pathways or Gene Ontology terms, in part because standard analyses are complicated by multiple diverged copies of most genes, including a large number of horizontally transferred genes. Transcript levels of these copies often showed independent changes across conditions, indicating evolution of extensive neo- and sub-functionalization in the *A. vaga* genome. In particular, many horizontally transferred genes and genes involved in DNA damage repair or antioxidant defense have copies that display different transcript patterns, indicating some may play specialized roles in resilience and resistance to desiccation stress. These results suggest that *A. vaga* could serve as an important natural system to study functional and structural genomics, with particular relevance for global change biology.

Study of the toxicity of TiO_2 and ZnO nanoparticles in rotifers from central and southern Mexico

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The use of titanium dioxide (TiO_2) and zinc oxide (ZnO) nanomaterials and its derivatives in personal care products by society causes an increase in the discharge of nanoparticles in the water. These nanoparticles cause adverse effects on biota causing environmental deterioration. Sunscreens are a type of personal care product, which contains TiO_2 and ZnO nanoparticles in its chemical formulation. Sunscreens are used indiscriminately in tourist areas. Therefore, the present study aims to study the acute and chronic toxicity, bioaccumulation and biomagnification of nanoparticles in rotifer species from the central (Aguascalientes) and southern (Quintana Roo) regions of Mexico. Zooplanktonic species from both regions were first established. Then, acute toxicity tests will be carried out, and the accumulation of nanoparticles in the lorica of exposed and unexposed organisms will be analysed by means of X-ray scanning electron microscopy (SEM). Exposure ranges are 0.01 to 100 mg/L. Our results

in nanoparticles showed that elemental composition of Zn and Ti are 52% and 60%, respectively. Our hypothesis is that the nanoparticles are incorporated into the zooplankton lorica that can be detected by SEM and X-rays analysis. Preliminary LC50 values are 1.007 mg/L for *Lepadella patella* and 53.86 mg/L for *Brachionus angularis*. This information is essential to verify our research hypothesis and contribute to the understanding of nanoparticle contamination in the environment.

Radiation resistance and DNA repair following exposure to desiccation and ionizing radiation are ancient and universal features in bdelloid rotifers: A new model for space research?

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Bdelloid rotifers have colonized any habitat where liquid water is temporarily available, tolerating desiccation and high doses of ionizing radiations thanks to their ability to repair DNA double strand breaks (DSBs). Here, these properties are compared among 9 bdelloid species collected from both mild and harsh environments, addressing the correlation between the ability of bdelloid rotifers to survive desiccation and their capacity to repair massive DNA breakage in a phylogenetically explicit context. Our analysis reveals that active DNA repair prevails in somatic cells of both desiccation-tolerant and desiccation-sensitive bdelloid species. Tolerance to desiccation and radiation are supported as ancestral features of bdelloid rotifers, with a few species like *R. macrura* and *R. rotatoria*, having lost this trait when colonizing permanent water habitats. Together, our results highlight the importance of studying distinct species with different ecological requirements within a specific clade to obtain a more comprehensive overview of desiccation and radiation resistance evolution among bdelloid rotifers. In as second part of the talk, we will present our latest transcriptomic data from cultivation of bdelloid rotifers on board of the International Space Station (ISS). Our project, RISE (Rotifer In SpacE) may

contribute significantly to our understanding of living in extreme environments and was designed with the intention to use bdelloids as a new model organism for space research. Impact of this peculiar environment on bdelloid rotifers will be discussed.

Host selection in the epibiont rotifer *Brachionus rubens*

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Epibionts such as rotifers live on the body surface of other organisms. When attached, the rotifers compete with their host over food and, in large numbers, damage the host's swimming ability and buoyancy. On the other hand, rotifers gain protection from predators and save energy. The invertebrate community of freshwater bodies is very dynamic regarding species composition and sex ratio, fluctuating over time and space. In addition, the spread of infectious diseases highly impacts the local invertebrate communities. These alterations often change the composition of potential host communities for rotifers. In the present study, we examined the preference of the rotifer *Brachionus rubens*, one of the most common epibionts of crustaceans of the order Cladocera, to different hosts. In a series of experiments, we examined the attachment preference of *B. rubens* to different species of the family Daphnidae. We also examined the attachment selection of *B. rubens* between males and females of the species *Daphnia magna*. Finally, we examined the attachment selection of *B. rubens* between healthy and infected *D. magna* with endoparasites. We found that *B. rubens* attaches in different numbers to different species of daphniids, with high attachment to the species *D. magna* and *Daphnia curvirostris* and no attachment to *Simocephalus vetulus*. We also found that *B. rubens* attaches more to male than to female *D. magna*, and that different species of *Daphnia* endoparasites influence rotifer attachment differently. Thus, the interactions among *B. rubens* and cladocerans could influence the community structure of invertebrates in fresh waterbodies.

Comparative ultrastructure of rotifer eggshells

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Species of Monogononta produce at least three kinds of eggs, each with a different shell: amictic, male, and mictic (diapause). As coverings, eggshells function to protect the embryo from the external environment but may have other functions including to facilitate transport (i.e., hydrochory, zoochory, or anemochory). The fine structure of rotifer eggshells is poorly known despite their important functions. Here, we investigate rotifers from 10 families that live in different environments (marine and freshwater) and have varied lifestyles (planktonic and sessile). We use transmission electron microscopy to characterize eggshell layers and determine whether there are commonalities among

eggshells within a species (amictic vs. mictic) and among related species and/or species from different environments and lifestyles. Our findings indicate that sessile species that live in protective tubes possess the thinnest amictic eggshells with minimal complexity. Planktonic species tend to have thicker amictic eggshells, but their thickness is not always related to having more shell layers. Resting eggshells tend to have the largest number of shell layers and are often the thickest shells. Many shells also possess spaces between layers that are interpreted as adaptations for floatation or anemochory.

Genome evolution and repair in the asexual bdelloid rotifer *Adineta vaga*

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Bdelloid rotifers are extremo-tolerant microscopic animals, surviving desiccation, freezing and high doses of ionizing radiation. During such stressful conditions, damages to macromolecules can accumulate, including DNA, that get repaired upon stress release. Besides, bdelloid rotifers are notorious ancient asexuals that engage in parthenogenetic reproduction without any males or syngamy. How this loss of sexual reproduction is affecting genome evolution in bdelloids remains unknown. Recent studies on new genome assemblies of the bdelloid *Adineta vaga* reported signatures of recombination but the underlying mechanism is still unresolved. We studied genome evolution by performing a mutation accumulation experiment (MA) including cycles of desiccation, and a genome repair experiment (GR) exposing *A. vaga* to ionizing radiation. Gains and losses of heterozygosity (LOH) were computed in the sequenced genomes of 46 lines. We detected in all MA lines, after 50 to 138 generations, a net reduction in heterozygosity. The genome spontaneously evolved towards homozygotisation in the absence of selection, without significant differences observed between lines submitted to desiccation. In the GR experiment, we observed an important plasticity of the genome with lines surviving large regions of LOH, large hemizygous deletions and complex breaks after high radiation doses. In GR most of the LOH is caused by deletions and repair of DNA damages, while in MA, LOH must be induced by endogeneous mechanisms such as a modified meiosis. Even in the absence of sex or stress, our study provides empirical evidence that homologous recombination plays a major role in the genome evolution of *A. vaga*.

Effects of SCCPs on the sexual reproduction of *Brachionus calyciflorus*: A populational and biomolecular study

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Short-Chain Chlorinated Paraffins (SCCPs) are ubiquitous, persistent chemicals which toxicity and potential impacts to aquatic ecosystems have been overlooked for many years. This study aims at determining the impact of these chemicals on rotifers. As sexual reproduction is often more sensitive to toxicants than amictic reproduction, this study focuses on endpoints describing sexual reproduction and on the impact on the gene expression of three receptors associated or suspected to be associated with reproduction the RXR, RAR and MAPR receptors. *Brachionus calyciflorus* was exposed to environmental concentrations of SCCPs (10, 100 and 1000 ng/L) and the impact on sexual reproduction (mixis rate, fertilization rate, resting egg production, gamete production) was assessed. Variations in the gene expression of RXR, RAR and MAPR were also studied through mRNA analysis. Our results show that SCCPs increase mixis rate but decrease fertilization rate, which leads to unaltered resting egg production. Impacts on mRNA levels of RXR, RA and MAPR were also noted and similarities between these biomolecular impacts and the reproduction study were observed.

The origins of reproductive isolation in diverging populations of *Brachionus plicatilis*

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The emergence of reproductive isolation within species stabilizes the differences arising among populations, hence being one of the factors in maintaining local adaptation and promoting speciation. Different processes and mechanisms are involved in isolation, and the isolation itself can influence other steps in the journey of evolutionary divergence. This contribution aims to present an overall view of the emergence of reproductive isolation in the rotifer *Brachionus plicatilis*, with special focus on pre-mating reproductive isolation and its genetic background. We studied different natural populations from the Iberian Peninsula diverging either due to restriction of gene flow associated to large geographical distances, or as a consequence of adaptation to local environmental conditions when in geographical proximity. By conducting no-choice mating assays, we quantified the degree of behavioural isolation in both geographically distant, as well as neighbouring, but environmentally diverging groups of populations. We also studied differentiation in genes involved in mate recognition. Albeit high dispersal capacity has been described in zooplankton species and stabilizing selection is acting on mate recognition traits, we found clear evidence for incipient behavioural

reproductive isolation in most of the mating assays. We also found relatively high diversity in genes for mating traits. Findings from both sets of experiments were more notable in populations diverging under different environmental conditions with ongoing migration than in geographically distant ones. We then examined whether these divergences map onto overall reproductive isolation, including the post-mating steps. While several population combinations showed some level of overall reproductive isolation, this was not correlated with the environmental or genetic predictors.

Phylogenetic annotation of rotifer heat shock protein 70 genes

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The 70 kDa heat shock proteins (HSP70s) and the constitutive members of the HSP70 family (heat shock cognates; HSC70s) are well-studied molecular chaperones, but accumulating evidence indicates that neither hsp70 nor hsc70 forms a monophyletic gene family, raising the need to reconsider the annotation strategy. We previously proposed a phylogenetic annotation of metazoan hsp70s based on their evolutionary history: HSP70cA/B for the cytosolic lineage, HSP70er for the endoplasmic reticulum lineage, and HSP70m for the mitochondrial lineage. In the present study, we firstly applied this strategy to the genome of *Brachionus plicatilis* sensu stricto, systematically annotating eight canonical hsp70s of this species. HSP70 genes from other rotifers such as *B. manjavacas, Asplanchna brightwelli*, and *Adineta vaga* were also included the analysis. This approach established using rotifers may be a new standard to annotate metazoan hsp70s as it has already been incorporated into *Drosophila melanogaster* hsp70s as aliases in the FlyBase.

Heat Shock Protein 40kDa variation in the *Brachionus calyciflorus* species complex

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Although the cryptic species concept has gained considerable importance in the recent years, cryptic species however, still pose a major challenge to ecological theories (e.g., co-existence theory, niche concept) due to the lack of morphological and physiological differences and a sympatric occurrence. The freshwater *Brachionus calyciflorus* species complex,

has lately been subdivided into *B. calyciflorus* s.s., *B. dorcas*, *B. fernandoi*, *B. elevatus*. Previous studies have shown that species within the *Brachionus calyciflorus* species complex exhibit different thermotolerances, with *B. calyciflorus* s.s. being heat-tolerant and *B. fernandoi* heat-sensitive. In addition, they show different gene expression patterns under different temperature regimes. Here, we used a transcriptome-wide SNP analysis to select candidate genes responsible for the differences in temperature adaptation between *B. calyciflorus* s.s. and *B. fernandoi*. We identified a Heat Shock Protein (HSP 40kDa) which shows not only strong differences in gene expression between the two species, but also pronounced structural (synonymous and non- synonymous) differences consistent over a broader geographic scale (Europe: Germany, Italy, Austria; America). Species-specific HSP 40kDa alleles were identified and within *B. calyciflorus* s.s shared alleles were found between Europe and USA. Selection tests confirmed signs of positive selection, but the identification of species-specific sites under selection failed due to the accumulation of weakly deleterious mutations. Additionally, the phylogenetic comparison of the mitochondrial COI and the HSP 40kDa uncovered a hybridisation event between *B. calyciflorus* s.s. and *B. fernandoi*, suggesting that a prezygotic isolation mechanism prevents the boundaries of niche differentiation from becoming blurred.

Does "form follow function" in the rotiferan genus *Keratella*: A meta-synthesis

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Most *Keratella* species are easily recognized by their dome-shaped, dorsal plate composed of a network of polyhedral units called facets, which are delineated by slightly raised ridges. The assembly of facets defines a species' facet pattern (FP), with the resulting structure resembling a geodesic dome. Previous researchers have sorted species into categories based on their FP, but the patterns have not been methodically analyzed. Additionally, while researchers have suggested that their strong lorica affords protection from certain predatory copepods, we really know little of the functional significance of FPs. Thus, our study had dual purposes. First, we tested the hypothesis that there is support for categorizing *Keratella* species into unique morphospace groupings based on their FPs. Second, using the structural analysis software SkyCiv©, we modeled FPs of *Keratella cochlearis*. This application allowed us to test the hypothesis that FPs provide resistance to physical stress. We found support for four FP categories, but not all available FP morphospace is occupied. Our SkyCiv analysis provided 'proof-of-concept' that *Keratella* FPs have a functional

significance. This was evidenced by the fact that reducing the number of facets comprising an FP was followed by a concomitant loss of structural reliability. We posit that FPs are an adaptation protecting *Keratella* from fractures to the lorica that may be caused by blunt force trauma. Such stresses may be incurred during predatory attack by copepods or while caught within the branchial chambers of daphnids. FPs are not limited to *Keratella*; they are present in other monogononts.

Using Oxford Nanopore MinION sequencing runs to identify genes from across a diverse set of monogonont rotifers

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Determining the genomic content of a diverse group of monogonont rotifers can provide an understanding of their genetic divergence, and information required to develop a more complete phylogenetic reconstruction. Although the number of monogonont genomes sequenced has recently increased, most of that work has been in the context of ecotoxicology and/or stress responses, and most have focused on *Brachionus* spp. To expand knowledge of rotifer genomic diversity, we sequenced eight non-brachionid monogononts using long read Oxford Nanopore MinION technology and found increased yields when coupled with QuantBio AccuStart Long Range Taq polymerase during PCR whole-genome fragment amplification. Subsequently, sequences were assembled using Flye, polished with Racon and then filtered to a minimum length of 1,000 bases. Braker was used to identify predicted coding regions that were then assigned functional annotations by Blast2GO. Quast and Busco were used to check assembly and protein calling, respectively. Taxa were sequenced in a single run to an average of 14 billion bases and with N50 read lengths ranging from 1,673 to 4,586 bases. Genome assembly sizes ranged from 39.0 Mb (*Hexrathra* sp.) to 216.9 Mb (*Plationus patulus*) with the largest scaffolds averaging 1.9 Mb across the taxa. Braker identified 11,162 to 36,699 proteins in the respective smallest and largest assembled genomes. Complete BUSCO scores averaged 92% across the taxa. Our work, along with previously published studies, will allow comparisons of multiple genetic pathways within Rotifera, including those related to reproductive processes (i.e., switching between mitosis and meiosis, amphoterism, and male dwarfism).

The transcriptomic response of the bdelloid rotifer *Adineta vaga* exposed to desiccation and ionizing radiation

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The extreme resistance to ionizing radiation in bdelloid rotifers is often correlated to their capacity to resist desiccation, with both stresses inducing reactive oxygen species, and damages to macromolecules and DNA. However, the underlying molecular genetic mechanisms involved both in desiccation and radiation resistance remain largely unknown. Here, we investigated the transcriptomic response of the bdelloid rotifer *Adineta vaga* to desiccation and to low (X-rays) and high (Fe) LET radiation. First, genes coding for antioxidants were observed to be constitutively highly expressed (Transcript Per Millions: TPMs>150; no differential expression observed between control and experimental conditions). Second, we found, specifically to rehydration (with or without irradiation), genes coding for late embryogenesis abundant proteins, heat shock proteins, and glucose repressive proteins being over-expressed and highly expressed (TPMs>150). In addition, we detected, in the common transcriptomic response to desiccation and irradiation, an over-expression and high expression (TPMs>150) of genes involved mainly in DNA repair, protein modifications (dephosphorylation) and proteolysis. These results suggest that their extreme resistance to radiation might be inherited from their capacity to resist complete desiccation. We specifically investigated the expression of DNA repair candidates in somatic *versus* germline cells through *in situ* hybridization. After exposure to X-rays, we detected transcripts of genes involved in homologous recombination repair within the oocytes, whereas genes coding for proteins involved in non-homologous end- joining and base excision repair pathways were expressed in the somatic syncytia. This study paves the way for functional experiments targeting promising candidates of radiation and desiccation resistance in bdelloid rotifers.

Rotifers functional diversity in a large fluvial Mediterranean watershed

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Rotifers, together with other zooplankton groups, have a fundamental position in water food webs and play an important role inside aquatic systems. Research on rotifers is vast and diverse, however, studies focused on their functional diversity are scarce compared with other fields. Indices like Functional Richness (FRic), Functional Evenness (FEve), Functional Divergence (FDiv) and Functional Dispersion (FDis) in comparison with traditional indices are based on the combination of species abundance and specific traits. In the present work we

evaluated these indices and their relationship to environmental and morphological variables from reservoirs located inside the Ebro watershed in Spain. Sampling campaigns were conducted during summertime from 2010 to 2019 in 64 reservoirs. Rotifer community in Ebro watershed was composed of more than 100 species and different traits. The environmental variables significantly related to functional indices were total phosphorus, conductivity and phytoplankton, and depth and volume of basin among morphological variables. Thus, the use of these indices has the potential to detect changes in the environment through the time and characterize the communities in the waterbodies. The present research is the first work done at a large scale with a functional diversity approach and could be employed in different habitats.

Co-culture of *Brachionus* cf. *plicatilis* and marine copepods (*Apocyclops panamensis* and *Ameira* sp.)

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As fish larvae increase in size, their prey preference changes from rotifers to micro-crustaceans. Polyculture of zooplankton of different species and size classes would facilitate adequate availability of live food during the different developmental stages of fish. Here we compared the population growth of *Brachionus* cf. *plicatilis* alone and in mixed culture with the cyclopoid *Apocyclops panamensis* or the harpacticoid *Ameira* sp. All the three zooplankton species were isolated from a shrimp farm and cultured in the laboratory for more than a year at a salinity of 20g/L on the chlorophyte *Nannochloropsis oculata*. In fish farms, regular change of medium is costly and difficult. To test the effect of periodicity of medium change on the rotifers, we compared population growth in a medium which was changed regularly and under conditions where the medium was not changed during the entire study period. Experiments were conducted in recipients of 20ml or 200ml capacity. The culture medium was 100% replaced daily in the former but never in the latter. Total rotifers, their eggs, and copepods were enumerated daily. The peak population densities of *B.* cf. *plicatilis* were higher in the experiments conducted with treatments where the medium was replaced daily (~40 ind. /ml) as compared to those where the medium was not changed (~25 ind./ml). The population growth rates of rotifers in controls were similar to those co-cultured with copepods. *Apocyclops* and *Ameira* are not as voracious as other species of copepods, and therefore, rotifers can coexist with them. Our results indicate that *B.* cf. *plicatilis* and *Apocyclops panamensis* or the harpacticoid *Ameira* sp. can be effectively cultured together.

A ploidy shift between closely related lineages of the bdelloid rotifer *Habrotrocha ligula*

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Models of genome structure in bdelloid

rotifers have evolved over the past two decades. Interpretations have included diploidy with anciently divergent former alleles, degenerate tetraploidy with an ancient genome duplication and closely similar pairs of co-linear chromosomes, haploidy with homologous copies as inseparable chromosome-scale palindromes, and most recently functional diploidy with paired homologous chromosomes nested in an older genome duplication. Each of these developments has informed parallel attempts to understand genome dynamics in these enigmatic invertebrates, including duplication, recombination, repair, gene conversion and inheritance. Here, we present results that further expand the range of potential structures and processes occurring in bdelloid genomes. We recently sequenced and assembled two genomes of the species *Habrotrocha ligula*, from cultured lineages that are close mitogenomic relatives but were collected on separate continents. One clone has a diploid-tetraploid genome similar to assemblies from other bdelloid species, but the other appears to be a triploid-hexaploid, with one extra homologous copy of each locus. We discuss potential mechanisms that could account for this difference and test these by analysing copy divergence within and between isolates, revealing some patterns that are noteworthy given the biogeographic origin of the animals. A rapid ploidy shift and inheritance of an odd set of additional gene copies invite reappraisal of broader assumptions and inferences about the structure and dynamics of bdelloid genomes.

Bdelloid rotifers in rock pools: An introduction to a rarely studied fauna

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Freshwater rock pools are unique habitats with an apparent structural simplicity – a depression in a rock filled with rain water – that often contrasts with the extremes of physical conditions experienced by their inhabitants. Rock pools may go through changes in water levels from full to dry during the course of a few days and temperatures from high (>35 °C) to freezing during the course of a year. They are inhabited by a small number of animal and protist species. Although bdelloid rotifers are common in rock pools, no surveys of rock pools specifically for bdelloids have been published. I have studied the bdelloid faunas of a number of small freshwater rock pools in western Turkey. These pools have volumes less than about 20 L with low water conductivities and are dry during summer months. The diversity of bdelloids adapted to persist in such habitats appears to be restricted to three genera (*Abrochtha, Adineta* and *Philodina*) and usually only one to three species may be found in one rock pool. The hydrological conditions and bdelloid species of rock pools are comparable to those of ordinary birdbaths. Birdbaths may serve as easily accessible artificial analogues of rock pools and provide opportunities, including *in situ* microscopic observations, for the study of bdelloids adapted to live under extreme conditions. A study of a birdbath in the author's garden that had four bdelloid species suggests that species cohabiting small rock pools may be spatially segregated.

Disentangling the physiological heat boundaries among species of the *Brachionus caliciflorus* species complex

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Cryptic diversity, i.e., species with considerable genetic disparity which manifest similar morphology, may also exhibit different physiological boundaries. Knowing the association between cryptic diversity and their physiological constrains may facilitate our understanding of organismal responses to climate change and enhance our predictions about biodiversity loss. Rotifers exhibit high levels of cryptic diversity. The freshwater *Brachionus calyciflorus* species complex, has recently been delimited into four species, *B. calyciflorus* s.s., *B. dorcas*, *B. fernandoi*, and *B. elevatus*. Based on previous observations about a sympatric occurrence where cryptic species succeed each other on a seasonal basis, we measured thermal tolerance as a bi- dimensional phenotypic trait affected by both the intensity and the duration of heat exposure. We found that clones belonging to *B. calyciflorus* s.s. sustained higher intensity of heat than clones belonging to *B. fernandoi*. We further used comparative transcriptomics to unravel shared and opposing patterns in gene expression between heat-tolerant (*B. clayciflorus* s.s.) and heat-sensitive (*B. fernadoi*) species under increasing temperature regimes. In both species, we identified key functional genes (e.g., Heat Shock Protein 90) for which gene expression was up-regulated with an increase in heat intensity. Therefore, these genes might be involved in increasing thermal tolerance. We also found a contrasting gene expression pattern between the two species in stress related genes. For these genes, gene expression was up-regulated outside of the optimum temperature for maximum population growth and down-regulated under optimum conditions. These genes can be further tested in other organisms and may provide a useful tool to assess vulnerability to temperature stress in aquatic environments in general.

Resting stages and spatial dispersal contributions to rotifers and cladocerans community structure

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Dispersal success is integral to survival of species in metacommunities. For many passive species like zooplankton, reliance on dispersal vectors is paramount for colonization of patches. Zooplankton species engage in dispersal in time (resting eggs) and space (overland). We performed

field mesocosm experiments simulating dispersal and colonization success of zooplankton via directional wind/air borne, resting eggs and biotic vectors in the vicinity of three ponds in an agricultural matrix. We applied a factorial design with live and dead sediment and with and without mesh cover. Mesocosms were sampled six times in 90 days. We hypothesize that: 1) Open mesocosms will attract biotic vectors and dispersal success will be more effective than in closed mesocosms. 2) The sediment harbors an efficient egg bank serving for rapid colonization of empty patches. 3) Cladocerans depend more on their egg-bank and biotic vectors than rotifers. We found variation in colonization rates between rotifers and cladocerans. The relative contribution of resting stages to dispersal and colonization success for both rotifers and cladocerans was considerable. Biotic vectors contributed to dispersal success especially for cladocerans. Wind/airborne dispersal was less efficient for cladocerans in relation to rotifers. Our study demonstrates that dispersal success is dependent on the mode of dispersal and the zooplankton group. Different dispersal vectors can generate distinct community structures influencing metacommunities and the combined effects of spatial dispersal and dispersal in time can lead to homogenization of isolated communities on small scales.

Evaluating toxicity of polystyrene microplastic on the rotifer *Brachionus calyciflorus* at short- and long-term exposure

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The increasing pollution of microplastics is of major environmental concern, particularly in aquatic ecosystems. In the present study, polystyrene (PS) spheres (1, 3 and 6 µm) were evaluated in a combination of short- and long-term toxicity experiments with the freshwater rotifer *Brachionus calyciflorus*. In the short-term experiments (24 and 96 hours), we employed silica (Si) spheres of the same size as a natural reference particle to distinguish between a particle and a plastic effect. The microplastic was fed together with food algae in varying food/non-food ratios. In the short-term experiments, food shortage was used as an additional stressor, while sufficient concentrations of food algae were available in the long-term experiment. We found no acute toxicity in the short-term experiments, neither for silica nor for PS. However, both particle types caused increased mortality and lowered reproduction after 96 hours compared to the particle-free control, with particle size playing a marginal role. In the long-term experiment, we found a reduced reproduction only for the 3 µm polystyrene sphere, which was similar in size to the food algae. Our results suggest dilution and particle effects rather than direct toxicity of polystyrene microplastics. We recommend the use of reference particles and the combination of short- and long-term experiments to investigate microplastic effects on aquatic organisms for proper risk assessments.

Ecotoxicity of pesticides and semiochemicals used for control and prevention of conifer bark beetle (*Dendroctonus* spp.) outbreaks

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Application of pyrethroid pesticides and semiochemicals are two treatments used worldwide to control conifer bark beetles (*Dendroctonus* spp.), but their residues can reach water reservoirs and water currents through run off and affect non-target organisms such as freshwater invertebrates. Therefore, we assessed the 48-h lethal toxicity, chronic toxicity (reproduction inhibition), and bioaccumulation of three pyrethroid pesticides (bifenthrin, cypermethrin, and deltamethrin) and two semiochemicals (verbenone and 3-methyl-2- cyclohexen-1-one) in two freshwater invertebrates: the cladoceran *Alona guttata* and the rotifer *Lecane papuana*. Bifenthrin was the most toxic of the five chemical compounds tested followed by deltamethrin and then cypermethrin, which was the least toxic pyrethroid for both species. Semiochemicals were far less toxic than pyrethroids, while verbenone showed higher toxicity than 3-methyl-2- cyclohexen-1-one for both species. For the rotifer *L. papuana*, the pyrethroid with the highest bioconcentration factor was bifenthrin, and for the semiochemicals it was 3-methyl-2-cyclohexen-1- one. For the cladoceran *A. guttata*, the pyrethroid with the highest bioconcentration factor was cypermethrin and for the semiochemicals it was verbenone. The pyrethroid with the highest body burdens both lethal and chronic was cypermethrin. Semiochemicals showed lethal and chronic body burdens 12- fold higher than pyrethroids and were therefore less toxic than pyrethroids. These results showed that the semiochemicals verbenone and 3-methyl-2-cyclohexen-1-one represent a safer tool for the freshwater invertebrates tested when compared with pyrethroid pesticides. Cypermethrin was the least toxic of the pyrethroids tested and therefore could be considered as a good candidate to control outbreaks of the conifer bark beetle.

Cryptic species in the U.S. Chihuahuan desert: A comparison of five widespread and commonly occurring taxa

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Many rotifer species believed to have cosmopolitan distributions are in fact complexes of cryptic species, wherein multiple species are morphologically very similar to one another but exhibit significant levels of genetic divergence. Here we compare rates of

cryptic diversification in five Chihuahuan Desert rotifer species: Bdelloidea, *Philodina megalotrocha* (87 sequences, 30 populations), and Monogononta, *Epiphanes chihuahuaensis* (49 sequences, 5 populations), *Euchlanis dilatata* (55 sequences, 28 populations), *Lecane bulla* (11 sequences, 9 populations), and *Plationus patulus* (17 sequences, 16 populations). We hypothesized that as these rotifers all occur within the Chihuahuan desert they should be experiencing similar ecological and evolutionary pressures and therefore have similar rates of cryptic speciation. Genetic divergence was determined through the following methods: Generalized Mixed Yule Coalescent (GMYC) and Automatic Barcoding Gap Discovery (ABGD) modelling for species delimitation, as well as haplotype diversity, and Most Recent Common Ancestor (MRCA) modelling based on COI gene sequences. Preliminary results suggest that target taxa have different rates of cryptic speciation present in their respective lineages. For example, most conservative results generated show no cryptic lineages found within *P. patulus* populations and the MRCA is genetically close to the tips of the tree whereas in *P. megalotrocha* 13 cryptic lineages were found and it also had the oldest estimated genetic MRCA. These results suggest that unique environmental and evolutionary pressures, such as differential rates of competition within each population or repeated founder events due to the temporal instability of their habitats, may be driving differential rates of speciation among these taxa.

Rotifer-heliozoan interactions: A population growth study

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Most predator-prey studies among rotifers are on asplanchnid-brachionid relationship. Other predators, including larval fish and crustaceans, are mainly tested using rotifers as live prey in aquaculture. In shallow water bodies, many different types of predators including heliozoans control the abundance and dynamics of rotifers. Heliozoans are predominantly freshwater, microbial eukaryotes. Armed with many stiff projections, the axopodia, heliozoans capture and engulf prey individuals, including rotifers. Though passive predators, they incessantly feed on both planktonic and benthic prey. In this work, we studied the population growth patterns of four rotifer species, *Anuraeopsis fissa*, *Brachionus calyciflorus*, *Brachionus havanaensis* and *Plationus patulus*, cultured separately and together with the heliozoan *Actinosphaerium* sp. Experiments were conducted in 10 ml reconstituted moderately hardwater containing green alga *Chlorella* (at a density of $1X10^6$ cells/ml) in Petri dishes. Initial density of each rotifer species was 1 ind./ml, and that of the heliozoan was 0.1 ind./ml. We quantified the abundance of the prey and predators in each test container daily, and replaced the medium. Our results showed that depending on rotifer prey availability, a single individual of heliozoan was able to reach a population density of 10

individuals within 12 hours through binary fission. The heliozoan took about 2 hours to engulf its rotifer prey fully. All four rotifer species declined significantly within a week when cultured with *Actinosphaerium* sp. Heliozoan density increased with increasing prey size. However, if any heliozoan did not capture a rotifer prey, its size was reduced and it died within 48h. Heliozoans also did not survive on the alga *Chlorella* alone.

Clonal erosion in rotifer populations

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Cyclically parthenogenetic rotifers may propagate clonally for many generations until external factors trigger sex and resting egg production. During such extended phases of clonal reproduction (which can occur during a growth season in the field, or during prolonged culture in the laboratory) genotypic diversity of populations is usually diminished. This process of 'clonal erosion' has been documented extensively by the use of neutral DNA and protein markers, but few studies have focused on how clonal erosion affects the distributions of phenotypic traits, and on the extent to which selection vs. drift contributes to the loss of diversity. In the present study, I am using experimental populations of the rotifer *Brachionus asplanchnoidis*, which are variable in genome size and initially consisted of 80-100 different clones, and used their genome size distributions as a marker to track clonal erosion. At the start of the experiments, high clonal variation of the populations was reflected in a broad, smooth, and unimodal distribution of genome size. During the experiments, these distributions progressively changed into more rugged shapes, and in some cases, only one or a few narrow peaks remained, suggesting that a few clones dominated the population at the end. Weekly samplings allowed to track the temporal dynamics of clonal erosion throughout the experiment, and experimental treatments (nutrient-limited vs. replete environments; different genetic composition of the initial population) informed about selection vs. drift as the force underlying the observed evolutionary change.

A survey of rotifer studies in karst habitats: A case study deep *vs.* shallow lakes of Balkan Peninsula

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Great share of karst area is situated in the Mediterranean region. Recently, due to

high turistic pressure and climate changes freshwater ecosystems, particularly lakes and ponds, undergo drastic, negative environmental changes. Unfortunately, rotifers are still understadied component of those threatened ecosystems, instead they play an important and pivotal role in the the foodwebs of both, deep and shallow waterbodies, and they are reliable indicators of the water quality, according to their traits: diversity, abundance, biomass, size and feeding structure. In this survey, rotifer traits across three types of threatened ecosystems were assessed: (i) deep karst lakes of Balkan Peninsula (Plitvice Lakes and Visovac Lake, Croatia; Shkodra Lake, Albania; Ohrid Lake, Macedonia); (ii) permanent shallow waterbodies in temperate climate (inland Croatia) and (iii) ephemeral waterbodies in Mediterranean climate (Adriatic Sea, Croatia). Overall, trends of deteriorated water quality were exhibited across studied ecosystem, reflected in decreased biodiversity, increased abundance of euryvalent rotifers (up to three species), decrease in the specimen's body size and transition of feeding preferences, from algivorous to detritivorous. In deep karst lakes still prevailed large-sized algivorous rotifers (genera *Synchaeta*, *Polyarthra*, *Trichocerca*), however with increased trophic state small-sized detridivores overtook domination. Abundance:biomass ratio indicated notable deterioration particularly in Mediterranean ponds with dominance of high abundance of small-sized detritivorous rotifer specimens. In shallow waterbodies of both climates submerged macrophytes enhanced diversity and water quality, while fish showed negative impact. Conclusively, results of studied ecosystems suggested importance of rotifers in their interactions with other zooplankters, aquatic organisms and environmental conditions as significant factor in the assessment of ecosystem functioning, as well as in the lake restoration.

Phylogeny and genetic variability of Rotifer's closest relatives Acanthocephala: an example from Croatia

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Acanthocephala or thorny headed worms is a group of obligatory endoparasites of Mandibulata and Gnathostomata which together with Rotifera form a monophyletic group referred to as Syndermata. Despite their close phylogenetic relationships, different lifestyles have led to different morphological and physiological characteristics of these two phyla. Although Acanthocephala is a relatively homogeneous group of parasites, considerable intraspecific variation within species can be observed. Here we present up-to-date status of phylogeny of acanthocephalans found in the freshwater fish in Croatia, including both the widespread species with broad range of hosts (e.g. *Pomphorhynchus laevis*) as well as the species confined to limited geographic areas with strong host specificity (e.g. *Dentitruncus truttae*). The sequences of three genes evolving at different rates (18S rDNA gene, COI gene and ITS region) were analysed in order to gain molecular insights into their phylogeny and genetic variability. 18S

rDNA was useful for determination of the phylogenetic position of understudied (e.g. *D. truttae*) or not morphologically precisely identified acanthocephalans (*Echinorhynchus* sp.), but was non-informative for intrapopulation variability detection. For the most examined acanthocephalans the genetic variability of COI marker was low (≤1%) while the highest values were observed for the sister species of the genus *Pomphorhynchus* (0-10.3%). The similar results were obtained for the genetic variability of ITS region, with the highest values observed within *Pomphorhynchus* species (0-11.3%). Our results are important not just for further phylogeographic studies but also for precise identification of acanthocephalan genotypes which can serve as bioindicators in freshwater ecosystems contamination studies.

The life cycle of two Antarctic bdelloid rotifers, *Philodina gregaria* and *Adineta grandis*

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Bdelloid rotifers are a key, widespread and abundant group of Antarctic microscopic invertebrates, with large populations reported from the microbial mats of several Antarctic ephemeral wetlands. As a first step to understand the trophic structure, energy flow, and matter cycling properties of the microbial mats and the ecological role of bdelloid rotifers in them, it is necessary to first determine the life history parameters of the bdelloid species present. However, due to the limited accessibility of Antarctica, only a few studies of Antarctic bdelloid rotifers are available. To address this need we collected two Antarctic species, *Philodina gregaria* and *Adineta grandis*, from the microbial mats of the ephemeral wetland on the Sôya Coast, East Antarctica and examined reproductive capacity at different temperatures. We found that *P. gregaria* reproduced over a temperature range of 3 – 20°C and produced an average of 15 ± 3 offspring/individual at 12°C and 0.07 offspring/day at 20 °C, and took an average of 20 ± 8 days to mature (the time to first reproduction) at 15°C. In contrast, *A. grandis* reproduced over a narrower range of 3–9°C and produced a total of 5 ± 1 offspring/individual at 9°C and 0.02 offspring/day at 6°C, and took an average of 40 ± 6 days to mature at 9°C. In conclusion, it was found that *Philodina gregaria* is more adapted to a wider range of temperatures than *Adineta grandis*.

Environmental selection on body size in the *Brachionus plicatilis* cryptic species complex

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The aim of the study was to compare the interspecific and intraspecific (= interclonal) body size response to heterogenic environment within the *Brachionus plicatilis* cryptic species

complex. We examined body size response of five species inhabiting 10 brackish ponds in the Mediterranean coast of Spain, to four environmental parameters: temperature, oxygen concentration, pH and salinity. The analysis of environmental characteristics selected salinity and oxygen concentration as the most important factors, while temperature and pH were less influential. Path analysis for clones without reference to species showed a positive effect of pH on body size and no effect of any other parameter. At the interspecific level, the body size response differed across species and parameters. The most consistent was the predicted smaller body size with lower oxygen concentration, while the predicted smaller body size at higher temperature was observed for one species only. Such a pattern could have been caused by interference of salinity, which did not affect body size directly, but affected the plastic response to thermo-oxygenic conditions. Under such conditions, oxygen seemed to be a more important driver of body size adjustment than temperature. We point out that different responses at among-species and within-species level may be caused by uneven distribution of species in the studied system, resulting from long-term-acting selective forces. We also suggest that oxygen concentration might have been one of the crucial environmental parameters causing niche differentiation within *B. plicatilis* cryptic species complex.

Dwarfism in male rotifers: Deciphering ecological and evolutionary drivers of sexual dimorphism

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Monogononts exhibit haplodiploid sex determination where unfertilized meiotic oocytes develop into sexually dimorphic, haploid males. Males may be of normal size or variably reduced in size and/or anatomy (dwarf). It has been postulated that dwarfism is a result of proportional dwarfism, and that dwarfism may be linked more to ecological conditions than phylogenetic position. We tested the hypothesis that ecology not phylogeny is driver behind male dwarfism by comparing the degree of dwarfism and complexity of the genitalia among groups with different ecological niches (planktonic, periphytic, benthic, sessile), and map these traits onto a preliminary phylogeny of the Rotifera. Dwarf males occur in 12 families across Ploima and Gnesiotrocha (40%), but there is no data on males of the remaining 18. We collected data from representatives of some of the remaining taxa to determine 1) whether dwarf males are present in all families (if not all genera), 2) whether male morphology differs across families and suprafamilial clades, and, using the phylogenetic tree, 3) where dwarfism has evolved. Planktonic males are often structurally reduced with rudimentary guts while benthic, periphytic, and males of sessile species may be of similar size or smaller than females and may have a complete gut. Of the families examined, 5 have everted penises (e.g., Brachionidae, Epiphanidae, Euchlanidae, Gastropodidae, Lecanidae), while 6 have evaginated penises (e.g., Asplanchnidae, Atrochidae, Flosculariidae, Mytilinidae, Notommatidae, Trichocercidae). The

importance of ecological drivers in male traits provides an explanation for why dwarf morphology is highly variable among species.

Biodiversity and new records of bdelloid rotifers from China

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Bdelloid rotifers are a group of microscopic invertebrates known for their obligate parthenogenesis and exceptional resistance to extreme environments. Although earliest research of bdelloid rotifers begins in 19th century in Europe, their diversity and distributions are poorly studied in China before 2017. In order to better understand the species distribution and diversity of bdelloid rotifers in China, we conducted a scienctific surveys of habitats (both terrestrial and aquatic habitats) from 13 provinces and regions of China, ranging from tropics to subtropics with a specific focus on poorly sampled areas (Oriental) from 2017 to 2021. A total of 113 morphospecies were found, of which, 66 morphospecies (including 1 family and 4 genus) are new records for China. In addition, we obtained 195 COI sequences of 17 morphospecies from China and constructed the Bayesian-based phylogenetic tree. A total of 21 cryptic species were found and indicated a high genetic diversity. Moreover, we compared the number of entities between generalist *Rotaria sordida* and specialist *Adineta beysunae* and indicated that habitat heterogeneity was a crucial triggering factor for the genetic diversity of bdelloid rotifers. This survey provides new data from a large region of China, enriching the knowledge of bdelloid biodiversity, and their global biogeography.

Multigenerational effect of microplastics on the demography of *Brachionus calyciflorus*

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Microplastics (MPs) are ubiquitous contaminants and stratigraphic indicators of the Anthropocene. Due to their low density, they float in the water column of freshwater systems. Since species of rotifers are the most common component of freshwaters, they interact actively with MPs, thereby experiencing potential ecotoxicological risk. These interactions can be diverse: involuntary ingestion of MPs by the non-selective filtration, mistaken as prey by invertebrate predators, transfer from lower trophic levels to higher by accumulating them, or causing nonreal satiety leading to death by starvation. The objective of this work was to evaluate the chronic effects of MPs (30 µm size) on two successive generations of a member of the *Brachionus calyciflorus* species complex under two temperature regimes (20 and 25°C). Standard life table experiments were performed using MPs at one concentration of 8 µg mL-1. Cohorts of twenty neonates were introduced into 20 ml medium in 30 ml capacity borosilicate transparent containers. Treatments, in

quadruplicate, consisted of the presence or absence of MPs in the test jars at two temperatures. Rotifer growth rates ranged from 0.55 to 1.05 d-1, depending on the treatment. The presence of MPs decreased the fecundity by 24% at 20°C and 20% at 25°C. The average lifespan of rotifers exposed to MPs in the F0 generation was statistically different from that observed in the F1 generation, indicating that pre-exposure of mothers may offer some protection to the offspring. The harmful effect of MPs on freshwater zooplankton species and their association with rising temperature levels due to global warming are discussed.

Large-scale patterns of mitonuclear discordance reflect the history of genetic exchange among species of a cosmopolitan hybridizing rotifer species complex

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Mitonuclear discordance refers to inconsistencies that may exist between phylogenies of mitochondrial and nuclear markers. This may have several reasons, such as a confounded taxonomy, interspecific gene flow or incomplete lineage sorting. The *Brachionus calyciflorus* species complex hitherto comprises four described species for which widespread mitonuclear discordance is observed between each pair of species. Studies indicated that hybridization is likely the most plausible cause. In this study, we investigated the phylogeographic structure of each of the four species across a large part of the Eurasian continent using the mitochondrial marker COI and the nuclear marker ITS1. More specifically, we investigated geographic patterns in the occurrence of mitonuclear discordances with the aim to unravel their origin and obtain an insight into past and current genetic exchanges among species. All four species were found to be widely distributed across Europe and China. Within species, COI clades and haplotypes are rarely shared between Europe and China indicating that gene flow between the two subcontinents has been very low to non-existent for a very long time (presumably millions of years). Mitonuclear discordances are found in both Europe and China at low frequencies. Introgressed COI haplotypes are found to be closely related or identical to those that still occur in the

ancestral species within the same geographic region. This indicates that currently observed hybridizations occurred relatively recently. Discordances do not seem frequent enough to erase phylogeographic structure.

Omega-3 long-chain polyunsaturated fatty acids are required for the formation and hatching of rotifer (*Brachionus plicatilis*) resting eggs

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Rotifers (*Brachionus plicatilis*) produce a smaller number of resting eggs (RE), when cultured with baker's yeast (*Saccharomyces cerevisiae*), instead of algae (*Nannochloropsis* sp.) (55 ± 2 vs. 1133 ± 132; $p<0.033$). All experiments were performed in triplicates per treatment, using 100 ml cultures and started with ~1,000 amictic females. Yeast lacks polyunsaturated fatty acids (PUFAs), but algae enhancing RE production contain EPA (20:5n-3) or DHA (22:6n-3). Since RE of algae-fed rotifers contained PUFAs, we tested whether PUFAs affect RE production and hatching. A higher number of RE was produced by yeast-fed rotifers treated with EPA (3,494.4±473), DHA (1,247.7± 545.1) or EPA+DHA (4,666.7±1,072.8) than controls (128.0 ±40.3). Differences were significant only for EPA and EPA+DHA ($p<0.0002$ for each) but not DHA ($p=0.155$), suggesting a minor role for DHA. More damaged RE were produced by yeast-fed rotifers ($p<0.001$, in all comparisons). A similar hatching success was found for intact RE from all treatments. Proteome profiling revealed differential abundance of proteins, including lipid metabolism enzymes, between fertilized females fed with yeast, algae, and those fed yeast with EPA, DHA, or EPA+DHA. Subsequently, we tested whether rotifers synthesize PUFAs by examining the effect of alpha-linolenic acid (ALA, 18:3n-3), a precursor of EPA and DHA in eukaryotes, on RE production and hatching. Yeast+ALA fed rotifers produced 10,211 ± 488 RE and their hatching success was 16.9±5.0%, compared with 1,975.6±488.0 and 3.8±2.3%, respectively, of controls ($p<0.00003$ and $p<0.0001$, respectively). Chemical analyses are in progress to identify and quantify the PUFAs which are present in these RE.

RotiferMiner: Development of an efficient tool to retrieve and organize GenBank sequences

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Rotifers have high levels of cryptic diversity and when compared to other freshwater invertebrates their true taxonomy remains unclear. DNA barcoding based on the sequence of specific genetic markers is a useful technique used when trying to resolve rotifer taxonomy. Databases, especially NCBI's GenBank, are used to store nucleotide sequences of rotifers, that are used in the analysis of their evolutionary history. GenBank contains a vast number of genetic sequences that will continue to grow. Research that relies on sequences downloaded from GenBank's website can be enhanced by having an efficient way to retrieve and organize them. We have developed a Python script that makes use of the NCBI Entrez system public API to assist in the retrieval and organization of DNA sequences of interest. This script downloads user specified genetic markers for each of the desired taxonomic groups in FASTA format, filters unwanted sequences based on a user defined exclusion list (e.g., sequence length and taxonomic classification), can combine downloaded sequences with internal laboratory records, and produces a list with counts-at the species level-of the specified genetic markers. This script can quickly allow researchers to identify species and/or genetic markers that have not been sequenced and prioritize them in the future.

The labellum of Rotifera - a newly described structure of the rotatory organ

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In the course of extended scanning electron microscopic studies across Rotifera, we have discovered a structural element of the rotatory organ that has escaped previous notice and for which we propose the name labellum. The labellum comprises two bilaterally symmetrical epidermal projections located ventrolaterally to the mouth opening and that are separated by a median cleft. Although fundamentally simple morphologically, the labellum nevertheless displays a high degree of morphological diversity with respect to the width of the median cleft, the form of the epidermis (e.g., smooth versus with distinct folds), and the form of its inner margins (e.g., smooth versus serrated). The labellum is variably present in rotifers and coincides largely with the monogonont taxon Transversiramida, where we confirmed its presence in at least one species from all its constituent families (Brachionidae, Euchlanidae, Lecanidae, Proalidae, Lepadellidae, Mytilinidae and Trichotriidae) except for Epiphanidae, where its presence is ambiguous. Apart from a convergent appearance in *Seison*, there is no evidence thus far for a labellum

in all remaining rotifer taxa. From this distribution, we hypothesize that the labellum might play a functional role in a grazing mode of feeding, which is characteristic of Transversiramida with their malleate mastax and might also apply to *Seison*, which apparently feeds on bacteria that grow on the integument of its host using its fulcrate mastax. However, too little is known currently regarding phylogenetic relationships within Monogononta as well as the feeding modes of its different species to test this hypothesis rigorously.

Study of the toxicity, bioconcentration and entry routes of lithium using the rotifer *Euchlanis dilatata* as a model organism

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Lithium is currently considered an emerging pollutant of interest to the scientific community regarding its environmental impact. However, the growing use of this mineral to meet the high demand for electronic items has generated few studies on its concentrations in environmental waters and on its effects on aquatic organisms. In this work we evaluate the toxicity of lithium in the freshwater rotifer *Euchlanis dilatata* using acute toxicity tests (24 and 48 h), sublethal reproduction and ingestion inhibition tests, fluorescence image analysis, X-ray diffraction analysis with scanning electron microscopy for the detection of microelements in the cuticle and atomic absorption techniques to determine the Bioconcentration Factor (BCF). This contribution integrated information on bioconcentration and body burdens, entry and accumulation sites. We determined acute values (24 h-LC50 = 2.5 mg/L and 48 h-LC50 = 0.18 mg/L) and chronic values of 0.73 mg/L and 3.93 mg/L for ingestion inhibition and growth inhibition respectively. Our results suggest a consistent mechanism in which lithium toxicity reduces ingestion by rotifer *E. dilatata* at low concentrations (EC50I = 0.73mg/L). However, the effect on reproduction (EC50r = 3.93 mgL-1) is observed at concentrations higher than the value of the 24 h-LC50 (2.5 mg/L). Determination of BCF in combination with acute and chronic data allowed calculation of acute and chronic body burdens. The information generated here could be used in studies focused on bioaccumulation and adverse effects at environmentally relevant concentrations. This information can be useful for the organizations in charge of regulating and protecting surface water resources.

The rotifer metacommunities of Mediterranean temporary ponds in the two hemispheres: A comparation between Eastern Spain and Central Chile

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Temporary ponds in Mediterranean areas, such as Eastern Iberian Peninsula and Central Chile, are known to undergo cyclic periods of flooding in autumn, and intense droughts during the hot and dry summer. These ponds show a wide spatial and temporal variability of their physico-chemichal conditions, but also regarding other characteristics as their geology, morphology, and hydrology. Here we analyze the environmental drivers and diversity patterns of two rotifer metacommunities across 30 mediterranean temporary ponds from Central Chile and from Eastern Spain. We sampled each pond thrice: after infilling, at the middle and before desiccation, and collected quantitative samples of rotifers. We measured and registered several local and regional environmental variables grouped in: limnological, biotic, landscape, hydrogeomorphometrical and climate variables. We aimed at determining whether rotifer communities exhibit neutral spatial structuring or they were structured by environmental features under niche-based control. We performed Redundancy analyses in order to define the relative importance of environmental factors in rotifer metacommunity structure. Rotifer metacommunities are represented by a total of 104 and 88 species in Chile and Spain, respectively. The factors explaining rotifer metacommunities varied between regions. In Chile, variables related with the landscape, physical and chemical characteristics, and aquatic vegetation dominated in explaining rotifer metacommunity, while in Spain climate and geomorphometrical variables had more influence. According to SIMPER analysis, species *Polyarthra dolichoptera*, *Lepadella patella* and *Hexarthra mira* in Spain and *Keratella cochlearis* and *Keratella valga* in Chile, contributed to main differences between regions and along the hydroperiod.

Multigenerational effects of environmental relevant concentrations of glyphosate and chlorpyrifos at single and combined toxicity to the marine rotifer *Proales similis*

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Studies assessing the interactive toxicity of pesticide exposure in marine organisms are scarce. Here we used the marine rotifer Proales similis to assess the single and combined toxicity of glyphosate (GLY) and chlorpyrifos (CPF) by acute toxicity testing and chronic multi- generational exposure experiments. TU50 of the mixture toxicity was estimated using eight toxic units (TU) by multiplying the LC50 values of each chemical. Rotifers were exposed to single and combined environmental relevant concentrations of GLY (0.001, 0.01, 0.100 and 1.000 mg/L) and CPF (0.0001, 0.0010, 0.0050, 0.0100 mg/L) for seven generations (F0 – F6). LC50 values were 35.20 mg/L (GLY) and 0.27 mg/L (CPF). TU50 was 0.30;

corresponding to 10.56 mg/L GLY and 0.08 mg/L CPF. The multi-generation study indicates that the higher concentrations of GLY and CPF, both single and combined, significantly decreased growth rates of *P. similis* consistently from F0 to F6. In most cases, GLY and CPF mixtures induced a synergistic effect. Our multi-generational study suggests that a mixture of GLY and CPF, as is likely to occur under real field conditions, increases the risk for marine invertebrates, such as rotifers.

Glycolitic enzymes of *Brachionus calyciflorus* as potential targets of glyphosate: an *in silico* approach

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Glyphosate affects several biochemical pathways and it has been reported to alter the energy allocation in aquatic invertebrates. Thus, this research was aimed to a) characterize *in silico* three glycolytic enzymes (hexokinase HK, phosphofructokinase PFK, and pyruvate kinase PK), and b) perform molecular docking of glyphosate and glycolytic enzymes. Amino acids sequences were obtained through tBLASTn from reported sequences in the Protein Data Bank. Transcripts and coding sequences (CDS) were identified with the aid of FGENES+ software. The subcellular location was predicted with LocTree3. Domains and motifs were identified with the online tool MotifSearch. The tertiary structure was assessed in Phyre2. Enzyme's ligands were inferred in the server 3DLigandSite, and the molecular docking with the aid of SwissDock and DockThor. We identified the CDS for the three enzymes, the number of exons (six to nine), the length of amino acid sequence (HK 553 aa, PFK 538 aa, and PK 836 aa). PFK and PK were located in the cytosol while HK was identified in the mitochondrial membrane. Motifs and domains of the enzymes correspond to those reported for homologous proteins. The three kinases possess ligand sites for ATP/ADP, carbohydrates, and metal ions. The molecular docking showed the interaction of glyphosate and glycolytic enzymes. Therefore, glyphosate may alter the energy metabolism of carbohydrates in rotifers when interacting with the glycolytic enzymes. In addition, this research provides primer sequences for further expression analysis, which will help to elucidate a likely pathway in the toxicity mechanism of glyphosate.

Survival and demographic responses of a *Philodina* sp. after exposure to ultraviolet radiation over multiple generations

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Prolonged exposure to ultraviolet radiation (UVR) can cause mutation of DNA, decreased lifespan, and reduced fecundity. Aquatic invertebrates inhabiting shallow waters are often unable to avoid UVR damage. Bdelloid rotifers demonstrate resistance to extreme environments and ionizing radiation. However, little is

known about their response to environmentally relevant levels of UVB radiation. We hypothesized that UVB exposure would negatively affect survival and selected life history characteristics (i.e., lifespan, net reproductive rate (R0), generation time (T)) at higher exposure intensities and over multiple generations. Bdelloids were collected from rock pools (< 0.5 m depth) in El Paso County, Texas, and used as a parental lineage (F0) for experiments. Within five days of collection, F0 rotifers were exposed to three UVB intensities (1.3, 3.7, or 5.0 W/m^2) for 2 h. After 48 h survival was assessed. Recovered individuals were then set aside; their broods (F1, F3) were isolated and cultured. Their offspring (F2, F4) were exposed to the same UVB treatment as the F0, and survival was recorded. Radiation negatively influenced survival as UVB intensity increased and with each generation (F0>F2>F4). In addition, life table analyses at 25˚ C were conducted using F1 and F5 individuals produced from females exposed to UVB. Preliminary results suggest that the 1.3 W/m^2 exposure increased lifespan by 118% and R0 by 250% compared to the control, but increased T by 45%. This may indicate that bdelloids have locally adapted to average regional levels of UVB radiation, however further analyses are still needed to confirm these results.

Quantification of metals in rotifers from five sinkholes of Quintana Roo, México

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The Yucatan peninsula has a karst aquifer, leading to sinkholes, which provide ecosystem services including drinking water, ecotourism, and cultural activities. In the last 10 years, anthropogenic activities have been increasing across the peninsula, causing infiltration of leachate and pollutants into the aquifer. To study the bioaccumulation of metal on the karst aquifer biota, rotifer abundance and richness were quantified in five cenotes at two seasons (rainy and dry), and the metals Al, Ba, Pb, Br, Cr, Cd, Fe, Cu, Li, Ni and Zn were measured using inductively coupled plasma atomic emission spectroscopy. Rotifers were collected and pooled for each site and season (40-297 rotifers per pool) to quantify metals and calculate bioaccumulation factors (BAFs). The species that were identified were: *Lecane bulla, L. quadridentata, L. leontina, L. luna, L. cornuta, L. ludwigii, L. hamata, Keratella americana, K. cochlearis* and *Brachionus havanaensis*. In the rotifer pools from the dry season concentrations of Fe and Zn were 0.006-0.007 mg/L and 0.003-0.004 mg/L, respectively. In the rainy season Fe, Li, and Zn were 0.0075-0.009, 0.0058-0.007, and 0.0027-0.004 mg/L, respectively. No other metals were detected in the rotifer pools, although Fe, Li, and Zn were detected in water samples at concentration of 0.004-0.393, 0.006-0.008, and 0.003-0.064 mg/L, respectively.

Effect of live food enriched with vitamin B complex on the population growth of *Asplanchna sieboldii*

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Rotifers are important live feed for fish larvae, crustaceans, mollusks, and other aquatic predators. In aquaculture, the enrichment of rotifers enhances their functions as live food. Vitamins are among the many products used to enrich live feed; however, on a large scale, the use of purified vitamins can be expensive. Our objective was to test the commercial grade vitamin enrichment of the prey *Brachionus calyciflorus* on the demography of the predator *Asplanchna sieboldii*. Daily 10µL/L of vitamin B complex was added to the *B. calyciflorus* culture for enrichment and its population growth and egg ratio were determined. We also compared the population growth of *A. sieboldii*, at three *B. calyciflorus* densities of the enriched prey (2.5, 5 and 10 ind./mL) and one treatment where only the vitamin was added to the test containers with the predators and prey. All experiments were conducted in quadruplicate. The population growth of B. *calyciflorus*, was enhanced by the addition of vitamins to the culture medium. Population growth rates of *A. sieboldii*, were significantly higher on the enriched *B. calyciflorus* as compare to the non-enriched prey at a density of 5 ind./mL. Low-cost enrichment of prey could be an alternative for live feed culture in aquaculture and will result in improved survival and growth of edible and ornamental aquatic species.

Environmental unpredictability and incomplete induction of sexual reproduction in rotifers

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Environmental fluctuations are a core feature of most habitats, having important ecological and evolutionary consequences. When fluctuations occur rapidly and unpredictably, organisms are expected to adapt via bet-hedging strategies. Facultatively sexual rotifers inhabiting water bodies in Eastern Spain are a good study model for this topic. The Mediterranean climate is characterized by strong random variations in environmental conditions. The persistence of rotifer populations during unsuitable periods requires life-history traits that match such environmental variation. *Brachionus plicatilis* populations are temporary, as they have to colonize the water column yearly during the planktonic growing season. The sexual phase of the life cycle is associated with the production of diapausing eggs that allow survival across growing seasons. Our aim was to address whether *B. plicatilis* populations diverge adaptively by adjusting the induction of their sexual phase to the level of unpredictability in their environment. Previous observations in natural and laboratory populations have shown that, once induced, sexual reproduction never is complete because both asexual and sexual reproduction continue to occur simultaneously. Here we estimated the

proportion of sexual daughters produced by individual females (i.e., sexual reproduction ratio) in a dynamic life- table experiment, using clones from two natural populations of *B. plicatilis* with contrasting levels of environmental unpredictability. We expected a negative relationship between the sexual reproduction ratio and environmental unpredictability. Results showed an incomplete response to sexual reproduction induction. However, no relationship was found between this adaptive strategy and the level of habitat unpredictability.

Predatory rotifers and fish models to assess nutritional quality of the prey *Brachionus calyciflorus*

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Corn is a staple diet in Mexico. For its use, the nixtamalization process (treatment with $CaCl_2$) is necessary, which improves its nutritional quality and digestibility. During this process, wastewater, known as nejayote, is generated. Nejayote contains high quantities of organic particles composed of proteins, lipids and carbohydrates. Since brachionid rotifers feed on particulate organic matters, nejayote can be used as a rich medium for their culture. However, nutritional quality of rotifers grown on nejayote requires further evaluation. In this study, we quantified the population growth of *Brachionus calyciflorus* fed different concentrations of nejayote. *B. calyciflorus* raised on nejayote was offered as prey (2.5, 5 and 10 prey ml/L) to the predatory rotifer, *Asplanchna sieboldii* to study its demographic responses. In addition, we also fed fish larvae (*Poecilia reticulata*) with *B. calyciflorus* raised on nejayote to study the somatic growth rates. The results showed that *B. calyciflorus* grown on 16% concentration of nejayote had significantly higher population growth rates as compared to 8% level or the controls (*Chlorella vulgaris*, 0.5x106 cells.ml/L). *A. sieboldii* fed *B. calyciflorus* cultured on nejayote at 16% also showed higher survival and reproductive rates as compared to other treatments. Somatic growth rates of larval *P. reticulata* fed B. *calyciflorus* cultured on 16% concentrations of nejayote were higher than on other treatments. We discuss the possibility of using *Asplanchna* bioassays as a surrogate for larval fish assays in the evaluation of nutritional quality of prey items in aquaculture industry.

Population genetics and dispersal of *Euchlanis texana* via anemochory in the Chihuahuan Desert

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Desert ecosystems present challenges for aquatic organisms because habitats are fragmented, both by space and time. Previous studies have shown that diapausing stages of rotifers can travel 10's to 1000's km during wind events. We used

the rotifer *Euchlanis texana* as a model species to investigate the influence of wind dispersal on gene flow and population genetics in the Chihuahuan desert. We hypothesized that dust storms facilitate gene flow from source populations in Mexico to habitats in the Trans Pecos region via delineated wind corridors. To test this hypothesis, the genetic diversity of populations from both inside and outside of the dust corridor were compared. The corridor was reconstructed using data from HYSPLIT trajectories of >20 dust events over 5 years. Genetic variation in the COI gene among populations from inside and outside of the dust corridor was analyzed. Moreover, isolation by distance, FST analysis, and a Random Forest classification algorithm will be used to investigate potential gene flow and population structure in the region. Preliminary data indicated 0 – 0.48% (mean: 0.20%) genetic divergence in the COI gene for the populations from inside the corridor and 0.8 – 2% (mean: 1.3%) for the populations from outside the corridor. Additional populations from inside the corridor and an additional marker (i.e., ITS) will be included to provide confirmation of our initial findings. Investigating anemochory's role in gene flow in desert environments will help us further understand evolutionary and ecological processes in aquatic microinvertebrates inhabiting ephemeral systems.

Transgenerational gene expression in *Brachionus plicatilis* in relation to environmental predictability

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Non-genetic transgenerational effects on the ability to respond to sex induction have been demonstrated in *Brachionus plicatilis* as related to environmental predictability. Clones from more predictable environments do not respond to sex-inducing cues during a number of generations after leaving diapause. This effect is hypothesized to prevent the untimely induction of sex while there are chances to fully exploit the planktonic growing season. Notwithstanding, the molecular basis of this mechanism is still unknown. In this contribution the expression level of genes related to (1) sexual reproduction in rotifers and (2) epigenetic signaling mechanism were tracked along successive generations from diapausing eggs in clones of *B. plicatilis* populations inhabiting ponds with different levels of environmental predictability. Specifically, the 17-β-estradiol dehydrogenase (*est*) gene, implied in 17-β–estradiol hormone synthesis, and a DNA methyltransferase (*dnmt*) gene were chosen as candidates to study changes in expression level across generations. We hypothesize that the expression level of the *est* gene will increase across generations in more predictable ponds, while that of *dnmt* genes will decrease (demethylation is a common epigenetic mechanism allowing the re-activation of silenced genes). Results show an increasing expression of *est* across generations in clones from the more predictable ponds studied. The expression of *dnmt* gene was higher in more predicable ponds than unpredictable

ones, although no difference across generations was observed.

In silico characterization of the Krebs cycle enzymes in six *Brachionus* species

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Glyhosate affects several biochemical pathways and it has been reported to alter the energy allocation in aquatic invertebrates. Thus, this research was aimed to a) characterize in silico three glycolytic enzymes (hexokinase HK, phosphofructokinase PFK, and piruvate kinase PK), and b) perform molecular docking of glyphosate and glycolytic enzimes. Amino acids sequences were obtained through tBLASTn from reported sequences in the Protein Data Bank. Transcripts and coding sequences (CDS) were identified with the aid of FGENES+ software. The subcellular location was predicted with LocTree3. Domains and motifs were identified with the online tool MotifSearch. The tertiary structure was assessed in Phyre2. Enzymes ligands were inferred in the server 3DLigandSite, and the molecular docking with the aid of SwissDock and DockThor. We identified the CDS for the three enzymes, the number of exons (six to nine), the length of amino acid sequence (HK 553 aa, PFK 538 aa, and PK 836 aa). PFK and PK were located in the cytosol while HK was identified in the mitochondrial membrane. Motifs and domains of the enzymes correspond to those reported for homologous proteins. The three kinases possess ligand sites for ATP/ADP, carbohydrates, and metal ions. The molecular docking showed the interaction of glyphosate and glycolytic enzymes. Therefore, glyphosate may alter the energy metabolism of carbohydrates in rotifers by interaction with the glycolytic enzymes. In addition, this research provides primer sequences for further expression analysis, which will help to elucidate a likely pathway in the toxicity mechanism of glyphosate.

Rotifer-specific biopolymers: the Rotimer family

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Exploration of natural polymers produced by rotifers is a relatively untapped field of research. Here we present a recently discovered property of some rotifers (e.g., *Euchlanis dilatata* or *Lecane bulla*) to produce an exogenic biopolymer (Rotimer). The secretion of this viscoelastic and proteinous biomolecule is calcium-ion dependent and stimulated by micro-particles (e.g., insoluble carmine crystals, sand, micro-cellulose, or metal-epoxy beads). This forms a Rotimer-Inductor Conglomerate (RIC) in a dense web format. The RIC's ability for various molecular interactions and bioactivities offers special evolutionary advantages (e.g., food and egg fixation, or antiseptic effect). Using in vitro bioassays, we examined relationships between species-specific Rotimers and three neurodegeneration disorders (Alzheimer's,

Parkinson's- and prion diseases). The RICs have significant anti- and dis-aggregating effects on relevant neurotoxic proteins (e.g., beta-amyloids, alpha-synuclein and prions). In addition, RICs exhibit inhibitory effects on motility of human neuroblastoma, yeast, and algae cells. The combination of low toxicity and the potential for protection against human-type beta-amyloid aggregates in vivo such that RICs may have medical significance. In addition, the study of the Rotimer family may open new avenues of research on Rotifera as well as providing a novel approach/tool in the field of biopolymers.

The rotifer community in a heavily modified waterbody following ecological disasters

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In the Mediterranean region, climate change is expected to intensify droughts, turning shallow lakes into brackish temporary waterbodies, thus altering their biodiversity and functioning. Understanding the resilience of the zooplankton in temporary waterbodies can help to predict how changes in these ecosystems will affect the community structure and is critical for effective conservation and management strategies. In Lake Koronia (North Greece), many dramatic changes and events (e.g., extended drought periods, decrease in water volume, harmful algae blooms, bird and fish kills) have taken place during the last decades. The aim of the present study was to investigate the diversity of the rotifer community, and biotic and abiotic interactions prior, during and after ecological disasters. The present study comprised zooplankton data, published and new, from 6 sampling periods across 20 years. Overall, 26 rotifer taxa were identified, with 8 being the maximum number of co-occurring species at the same sampling. Generally, high abundances of rotifers have been recorded only when the cladoceran *Daphnia magna* and the copepod *Acanthocyclops robustus* were absent, due to competitive exclusion and predation, respectively. Results indicated that temperature, pH and salinity appeared as main drivers in shaping the zooplankton assemblage during the sampling periods. More specific, rotifers, presented mainly by *Brachionus* species, were the most frequent taxa recorded in high abundance along with increased salinity. Further analysis will reveal effects of drought periods and abiotic factors on the recruitment, distribution and diversity of zooplankton.

Comparing toxicant sensitivities within the *Brachionus plicatilis* species complex using ingestion rate as a sublethal endpoint

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This research presentation will compare differences in toxicity sensitivity within and among the recently named 15 species that previously were in the *Brachionus plicatilis* species complex. Due to its importance in the aquaculture industry, this species complex is the most extensively

studied rotifer group. However, a study to directly compare ingestion toxicity sensitivities within this species complex has not been completed. Therefore, we proposed to use a simple ingestion assay to investigate the differences in toxicant sensitivities. The ingestion assay is a sub-lethal test that can quickly evaluate toxicity. The following toxicants were picked to be analyzed: lead, cadmium, mercury, and endosulfan/methyl parathion. The closely related species include *Brachionus rotundiformis*, *B. manjavacas* (Russia), *B plicatilis* (Salebrejo), *B.* sp (Lost Lake), *B. rotundiformis* (Italy), *B. plicatilis* (Avpea006), *B. manjavacas* (Petta), *B. manjavacas* (Man), *B. plicatilis* (China), *B.* sp. (Japan S-type), *B. manjavacas* (Gaynor pond), *B. plicatilis* (Tokyo), *B. calyciflorus* (Gaines), and *B.* sp. (Nevada2). Results will demonstrate differences between the ingestion rates of the above closely related species.

Zooplankton traits in the brackish Vrana Lake near Biograd na Moru (Croatia)

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Vrana Lake near Biograd na Moru is a karst field filled with water and is the largest natural lake in Croatia, connected to the sea by a channel. It was declared as a Nature park in 1999, mostly because of the ornithological reserve in its area. Zooplankton plays a significant role in lake ecosystems, especially in determining the ecological status of lakes. The goals of the research were: (i) to determine the abundance, biomass and diversity of zooplankton; (ii) to study the impact of biotic and abiotic factors on zooplankton dynamics; (iii) to study the impact of fish predation on zooplankton dynamics. In the two years period (2018 and 2019), zooplankton samples were taken at three longitudinal stations. In order to analyze the dynamics of zooplankton several abiotic and biotic factors were observed: temperature, oxygen, conductivity, salinity, ph, transparency, nutrients, chlorophyll *a*, and fish predation. The research revealed a significant influence of temperature, salinity, dissolved oxygen, transparency as well as significant influence of ichthyofauna on the structure and dynamics of zooplankton. This study of planktonic zooplankton will provide insight into the trophic state of the lake, which will enable the determination of guidelines for further management of Vrana Lake and protection of this valuable and important habitat.

Rotifers of Plitvice lakes National park (Croatia, Europe)

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Plitvice Lakes National Park area belongs to the karstic region of the NW Dinarid Mountains, and has both surface and subterranean drainage systems. The basic phenomenon of the Plitvice Lakes is a

formation of a porous calcareous precipitate (tufa) that shapes a cascade system of 16 oligotrophic barrage lakes interconnected by tufa barriers, waterfalls and cascades, channels and rapids. The lake cascade descends from an altitude of 636 to 503 m a.s.l. over a distance of 8.2 km and is divided into two clusters: (1) the Upper Lakes situated on less permeable dolomite and (2) the Lower Lakes placed in a narrow canyon composed of very permeable limestone. Rotifer fauna of Plitvice Lakes have been investigated from the middle of 20th century, and 105 different taxa are known so far. Most of investigations were conducted in two largest and deepest lake Prošće and Kozjak. Dominant and constant planktonic species inhabiting these lakes are: *Collotheca mutabilis*, *Kellicottia longispina*, *Keratella cohlearis*, *Keratella quadrata*, *Gastropus stylifer*, *Ascomorpha saltans*, *Polyarthra* spp., *Synchaeta tremula*, and *Trichocerca similis*. At the beginning of 21th century rotifers from plankton, littoral and benthos were considered within longitudinal transport of seston. Also, rotifers in bryophytes and tufa sediment were investigated, as part of meiofauna. The most common rotifers within bryophytes and tufa habitats were bdelloid *Philodina roseola* and several species from genera *Colurella* and *Lecane*.

Rotifer assemblage in temperate *vs.* Mediterranean ponds

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Rotifers and planktonic crustaceans (cladocerans and copepods) occupy a central role in the food webs of shallow water bodies, connecting autotrophic (algae) with heterotrophic mode in the food webs. Differences between temperate-continental and Mediterranean climates affect variously interactions of abiotic and biotic factors. The aim of this study was to determine: (i) the diversity, abundance and trophic structure of zooplankton; (ii) zooplankton indices in comparison to the trophy state indices; (iii) a proposal for measures in the management and conservation of shallow water bodies in two different climates. In nine ponds, 48 species of zooplankton were determined, and rotifers dominated with 36 species. Habitat complexity, macrophyte coverage, and nutrient concentrations were indicated as significant factors in the structuring of zooplankton in shallow water bodies of both climate regions. Zooplankton in the temperate-continental climate was characterized by populations of higher diversity, while in the Mediterranean climate zooplankton was characterized by populations of lower diversity and higher abundance, particularly species of genera *Lecane* and *Brachionus*. Results of this study support the importance of zooplankton as a sentinel indicator in assessing the trophic and ecological status of shallow water bodies and provide a basis for determining guidelines in the conservation and management of these sensitive and endangered ecosystems.

Do littoral rotifers (Rotifera, Monogononta) follow Bergmann's rule?

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Bergmann's rule, which states that "body size varies inversely with ambient temperature, so that body size increases with latitude", is one of the fundamental rules of macroecology. The rule is especially significant now, when global warming is expected to reduce the body size of ectothermic freshwater animals. By comparing the body size of six species of rotifers (*Lecane closterocerca*, *L. hamata, L. bulla, L. quadridentata, Plationus patulus* and *Testudinella patina*) from the tropical waters of Costa Rica with the size of the same species in temperate climates (Masurian Lake District, Poland and Arkhangelsk province, Northwest Russia), and from artificially heated waters (Konin lakes, Poland), we checked how the stability of thermal conditions influences the size of the researched rotifers. All the studied species are littoral, so they live in rather good oxygen conditions and are less exposed to predator attack than pelagic species. We found the lowest rotifer weights of four species from Costa Rica, and two from the Konin lakes. The lower size of four studied species in tropical water bodies as compared to heated lakes could be the result of more stable thermal conditions in the tropics. In this study we have found that at least for some rotifer species Bergmann's rule may be implemented. However, the best approach could be to test the rule on organisms with similar behavior and ecology.

Seasonal dynamics of rotifers in a high-altitude tropical reservoir

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Reservoirs located in the central plateau of Mexico are subject to the rainy-dry seasonal dynamics regulated by precipitation patterns, and thus water level drastically diminishes at the end of the dry season. To recognize the effect of seasonality on rotifer dynamics, we monthly studied environmental variables and rotifer diversity from August 2018 to July 2019 at eight sampling ephemeral waterboddies in the Taxhimay Reservoir (State of Mexico). According to the principal component analysis, Secchi transparency, conductivity, and chlorophyll *a* explain most environmental

variation throughout the year. We found 26 species of rotifers belonging to 16 genera and 12 families where *Polyarthra dolichoptera, Keratella cochlearis, K. americana, Conochilus unicornis* and *Synchaeta pectinata* were dominant species throughout the year. Rotifer abundances were higher in the middle of the rainy season (August), reaching 10066 ind.L^{-1}, while the lower abundances occurred in the cold-dry (December) season with 1362 ind.mL^{-1}. Diversity of Shannon ranged from 1.64 to 3.85 with lower values during the cold-dry season and higher in the rainy season. We also registered low abundances of cladocerans with a dominance of small size taxa such as *Bosmina* sp and *Ceriodaphnia* sp. High rotifers abundances were related to the eutrophic condition of this reservoir and the low presence of big size cladocerans due to fish predation.

Rotifer *Lecane inermis* as an alternative living food for small fish larvae

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In the fish farming main food supplies are mostly crustacean larvae *Artemia*, rotifer *Brachionus* and specialized artificial feed. However, each of these items has some limitations. A potential alternative is applying live rotifer specimens of *Lecane inermis* which are small, fragile, very flexible and easy to digest. Main advantages of culturing this rotifer species are: cost and space saving due to the lack of aeration requirement as opposed to *Brachionus*, high abundance of rotifers reached in small culturing vials, and also, easy to use for feeding the rotifers dry food rich in fatty acids, vitamins and minerals. Experiments were conducted on the fish hatchlings of *Danio rerio*. In the treatment, larvae were fed with rotifers *L. inermis* and in control artificial food was used. The daily requirement of rotifers per larva was estimated basing on decreasing rotifer density, whereas the length of the larvae was obtained from video still frames. At the end of the first part of the experiment an average percentage of surviving larvae fed exclusively with rotifers and fed with artificial food was 93% and 24%, respectively. To the end of experiment 71% of larvae fed with live rotifers survived in comparison to only 16% of those fed exclusively with artificial food. The larvae fed with *L. inermis* were notably more fit and agile than those fed with artificial food. Conclusively, results indicted rotifer *L. inermis* as suitable alternative food for the early developmental stages of *D. rerio*.

A combination of host ecology, habitat, and evolutionary history explains differences in the microbiomes associated with rotifers

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The holobiont concept places emphasis on the strict relationship between a host and its associated microbiome, with several studies supporting a strong effect of the quality of the microbiome on the host

fitness. The generalities of the holobiont have been questioned for several invertebrates, including zooplankton. Here we assess the role of host ecology, habitat, and evolutionary history to explain the differences in the microbiomes associated with rotifers, across a broad taxonomic spectrum and from different habitats. The analyses of 93 rotifer- associated microbiomes from 23 host species revealed a combination of effects, with a significant correlation between host phylogenetic distances and differences in microbial composition of the microbiomes, a pattern in line with the expectations of phylosymbiosis.

A georeferenced dataset of Italian rotifer records

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Biodiversity patterns are driven by different environmental variables at different spatial scales. However, data to analyse such trends in rotifers are not available, notwithstanding several decades of faunistic studies providing species lists on such animals. One way to partially overcome such a problem is to gather all the published records of rotifers for specific areas in order to have more data available for further analyses. Here I present the results of such a detailed literature survey for Italy. I found approximately 400 published papers, dating from 1838 to 2021, reporting a total of more than 15.000 records in Italy for about 1100 taxa at the species and subspecies level. We aim to make the data set publicly available for further studies on biodiversity.

Effect of the presence of a large-sized cladoceran on the populations of three brachionid rotifer species under different trophic conditions: A mesocosm experiments

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Rotifers and cladocerans interactions in freshwater ecosystems are of considerable interest. Due to their large size, cladocerans, are usually better competitors for food resources than rotifers. However, at a higher trophic state, rotifers could dominate over cladocerans. Therefore, we hypothesize that rotifers' ability to compete with cladocerans increases under extreme eutrophic conditions. We experimentally analyzed in situ the ability of *Brachionus calyciflorus, Brachionus caudatus* and *Keratella americana* to survive, and co-exist, in mesocosms with *Daphnia pulex*, in three tropical adjacent shallow lakes of different trophic state (mesotrophic, eutrophic, and hypereutrophic conditions) located in Central Mexico. For this purpose, weekly samples were collected, and rotifer's density and population growth measurements were carried out from two outdoor semi-enclosure mesocosms and a control per lake. After adding the cladoceran, *B. caudatus* under mesotrophic conditions was outcompeted, while *B. calyciflorus* and *K. americana* under

hypereutrophic conditions were not affected by *D. pulex*, and with non-significat differences with controls. In conclusion, *D. pulex* addition exhibited a weaker population response over brachionid rotifers under high-level productivity conditions, where persistent toxic cyanobacteria blooms were recorded. As opposed to that under moderate trophic conditions *D. pulex* was a stronger competitor over rotifers. Brachionid rotifer's competitive ability is predicted to be strongest in eutrophic and hypertrophic conditions, where filamentous and colonial cyanobacteria dominate, usually inedible by most zooplankton (especially larger cladocerans), favoring small-bodied rotifer species.

Rotiferan *ParaHox* gene expression indicates a conserved role in patterning of the nervous system

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Three genes, *Gsx*, *Xlox* and *Cdx*, form the *ParaHox* class of homeobox genes and are often organized in a small genomic cluster. Arisen from a duplication event along with the *Hox* gene cluster, *ParaHox* genes also frequently exhibit expression domains along the antero-posterior axis of various body plans. An evolutionary conserved role of this gene class in patterning of the bilaterian gut, dating back to the origin of Bilateria, has been proposed. *ParaHox* orthologs of the "anterior" gene *Gsx* and the "posterior" gene *Cdx* were initially isolated from *Brachionus manjavacas* by PCR. Apparent absence of an ortholog of the "central" gene *Xlox* in different gnathiferan taxa was validated by search of genomic data for *B. manjavacas* (Monogononta), *Adineta vaga* (Bdelloidea), *Seison nebaliae* (Seisonida) and *Pomphorhynchus laevis* (Acanthocephala). Genomic mapping shows a dispersed cluster, with *Bm-Gsx* and *Bm-Cdx* separated by 4.425 megabases. In contrast to the hypothesis that *ParaHox* expression would be associated with patterning of the gut, analysis of expression of *Bm-Gsx* and *Bm-Cdx* during embryogenesis, visualized by *in-situ* hybridization, indicates neuronal expression of *ParaHox* genes in *B. manjavacas*. *Bm-Gsx* exhibits distinct expression in FMRFamide-positive neurons connecting the caudal ganglion to the foot as well as two cells with neuronal characteristics in the region between mastax and stomach, possibly belonging to the stomatogastric nervous system. *Bm-Cdx* is expressed in the FMRFamide-positive cells forming the wall of the bladder. Integrating *ParaHox* expression in basally branching metazoan lineages, these results support an original role of *ParaHox* genes associated with the nervous system.

Effects of fish introduction on zooplankton community in an agricultural lake

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The study was conducted in the spring of 2018 in Lake Jošava (Eastern Croatia) before and after stocking with common carp (*Cyprinus carpio*) was conducted. The aim of the study was to determine whether fish stocking affected the composition of planktonic and periphytic communities at different sites (pelagic P1-P4 and littoral L1-L4 zones) of the lake. Samples were collected for community composition analysis (zooplankton, phytoplankton, periphyton). A significant difference in physicochemical parameters was detected using the one-way ANOVA. Water depth, transparency, and total phosphorus concentration differed significantly between sites. The qualitative composition of the periphyton revealed a wide diversity of taxa from the *Chrysophyta* and *Chlorophyta* groups found. Species from the same groups were also most represented in the qualitative composition of the phytoplankton. Rotifers did not change significantly before and after restocking. The most abundant rotifer groups were *Keratella* and *Brachionus*. A quantitative difference was observed between the pelagic and littoral sites throughout the study, with a large proportion of biomass at the second pelagic site (P2) two weeks after restocking. There was an obvious trend of change within the dynamics of the zooplankton groups in relation to the timing of stocking. The lowest number of rotifers was recorded one week after fish stocking. After one month of stocking, the abundance of rotifers and crustaceans increased, indicating reduced fish predation.

What do rotifers do in winter?

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Most research on zooplankton communities has focused primarily on the growing season, and their changes during the winter period have been poorly studied. The objective of this study was to examine changes in zooplankton and diatom communities during winter in an alluvial floodplain. The study was conducted weekly in Lake Sakadaš and Čonakut Channel in Kopački Rit Nature Park (Croatia) during winter 2019. Samples were taken for zooplankton and diatom communities, chlorophyl-a and water chemistry analyses. The abundance of rotifers changed significantly (one-way ANOVA $F=7.3$, $p=0.01$), with the highest abundance (328 ind/L) recorded at the end of the study when temperature increased significantly ($F=24.35$, $p<0.001$). The community was characterised by low diversity and high dominance. The predominant species were *Synchaeta tremula*, *Polyarthra vulgaris* and *Brachionus calyciflorus* species complex, whose co-dominance alternated. According to water chemistry analyses, the sampled sites were eutrophic to hypertrophic, but there were very few diatom species on which rotifers could feed. The environmental parameters that had a strong influence on rotifers were water temperature ($r=0.67$, $p=0.02$) and oxygen concentration ($r=0.48$, $p=0.043$). These results provide information about the dynamics of the winter plankton and show which environmental parameters are significant for the development of rotifers in winter.

The environmental impact of the COVID-19 pandemic: the effects of nanoscale gold colloids from rapid COVID-19 test strips on the planktonic rotifer *Brachionus calyciflorus*

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The ongoing global COVID-19 pandemic continues to have an enormous, widespread impact on human health and society. Its effects are being felt both mentally and economically. However, the consequences of the pandemic on the environment have been largely ignored and mostly concern the increase in microplastic pollution from disposable masks. Equally important as masks in controlling the pandemic, however, have been rapid COVID-19 test strips that are based on nanoscale colloidal gold as immunosensors. After usage, the tests can enter the environment via normal household waste, where the gold colloids (AuColloids) can be washed out by sewage or precipitation to potentially enter aquatic habitats. There, zooplanktonic organisms such as rotifers are exposed to and can ingest the suspended AuColloids. Using the limnic rotifer species *Brachionus calyciflorus*, we investigate the potential impact of its uptake of AuColloids that we obtained from 35 Hotgen Coronavirus 2019-nCov2 Antigen Tests (Beijing Hotgen Biotech Co., Ltd) and released in 10 ml milliQ water. We used ultrastructural analyses to investigate whether the AuColloids, like other gold nanoparticles, are ingested and internalized within the cells of the rotifers. Additional observations reveal the effect of three AuColloids concentrations on the mortality and reproduction rates of exposed rotifers. Altogether, our findings provide information about the extent to which the COVID-19 pandemic may be putting additional stress on aquatic microorganisms through increased nanoparticle pollution.

Effect of the surfactant sodium dodecyl sulphate on the morphology and population growth of *Brachionus havanaensis*

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Among a wide variety of pollutants, detergents cause serious problems to the aquatic environment including foam, reduction of dissolved oxygen, and release of phosphorus into the ecosystem. Due to the increased demand to meet the requirements of growing human populations, chemical industries globally produce about 12x106 tons of detergents per year. Directly and indirectly, the residues of these compounds reach

wastewater treatment plants. However, they are rarely completely eliminated. Sodium dodecyl sulphate (SDS) is an anionic surfactant present in many detergent formulations. *Brachionus havanaensis* is one of the most common rotifer species in Mexico. We tested its morphology and population growth when exposed to different concentrations of SDS. Based on a previous LC50 data we selected 6 concentrations (0, 0.625, 1.25, 2.5, 5 and 10 mg/L) of SDS for chronic testing. The population density of *B. havanaensis* was significantly reduced when exposed to the SDS concentration as low as 0.625 mg/L. Depending on the concentration, rotifers exposed to SDS showed some alterations in the lorica morphology especially at 5 and 10mg/L of the surfactant. In this work we discuss the results of the ecotoxicological effects of sodium dodecyl sulphate, highlighting the importance of using rotifers as a study model.

Characterization of Ligase E – a non-metazoan gene acquired by bdelloid rotifers and upregulated following irradiation

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Bdelloid rotifers are extremotolerant animals that can survive in harsh environments such as semi-terrestrial habitats, prone to regular episodes of drought. In addition to their remarkable ability to withstand complete desiccation or freezing for a long period, they can resist to high doses of ionizing radiation that are lethal to most organisms. At the cellular level, prolonged exposure to these genotoxic stresses induces the production of reactive oxygen species, causing protein and DNA damage, including single- and double-stranded DNA breaks leading to complete genome fragmentation. Upon stress withdraw and rehydration, bdelloid rotifers of the species *Adineta vaga* have the capacity to reassemble their fragmented genome and resume their activity. This species is characterized by an enhanced DNA repair ability and carries multiple copies of most DNA Damage Response (*DDR*) genes that are present in metazoans and non-metazoans. Remarkably, a homolog of the prokaryotic DNA ligase E (*LigE*) acquired through horizontal gene transfer by bdelloid rotifers is upregulated post-irradiation. In rotifer cell extracts, *LigE* appears to be the major contributor to the ligation of DNA breaks in vitro. Moreover, its heterologous expression in human cell lines significantly improved their radio- tolerance. We started to assess in vivo the role of *LigE* with the contribution of other DDR factors in DNA repair in *A. vaga* and human cells to get novel insights into the molecular mechanisms underlying radio-resistance.

Terrestrial bdelloid rotifers from humid area in north Algeria

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The bdelloid rotifers from terrestrial habitats and a wetland at two national parks in Algeria were studied. A total of 84 samples belonging to different habitats (soil, bark, plant, lichen, and moss) were collected at a high altitude (1100-1520 m) from The National Park of Chrea (NPC) and The National Park of Theniet El Had (NPTEH), and examined. Twenty-five taxa belonging to twelve genera and three families (Adinetidae, Philodinidae and Habrotrochidae), were recorded in the different samples. Species richness for each sample ranged from 2 to 16. The results of the analysis of variance (ANOVA) showed significant differences in species richness between habitats (ANOVA: $F_{5, 84} = 12.05$, $P \leq 0.0001$).

Little rotifer tales from the Middle East

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Israel extends over a relatively small area, which features Mediterranean, arid and semi-arid climate regimes with diverse meteorological parameters. Ephemeral ponds in this area are of high importance, because they are particularly impacted by climate change and urbanization. Rotifers have drawn little attention in many freshwater biodiversity surveys in Israel, likely due to their small size and the non-planktonic habitat of many species. To assess the monogonont rotifer biodiversity of this unique area, we rehydrated 20 g of sediment collected from 30 ephemeral ponds across Israel and incubated them at 20°C under a photoperiod of 12:12 h light:dark. Samples were filtered and screened for rotifers every week, for a period of four weeks. We found 41 species from which 26 are reported for the first time from Israel, considerably increasing the known diversity of rotifers in the area and expanding the known distribution of some species such as *Rhinoglena ovigera*. We found differences in the diversity, species richness and abundance of rotifer communities in a relatively small geographic area, which indicates that the community of microscopic organisms is affected by local environmental conditions rather than distribution limitations. The highest diversity was reported in the Mediterranean coastal plain, which is characterized by low altitude and long hydroperiod. On the contrary, the highly unpredictable desert ponds, which are characterized by high altitude and short hydroperiods, yielded the lower biodiversity. However, they yielded a unique rotifer composition with species such as *Hexarthra mira* and *Cephalodella catelina* recorded mainly from the desert area.

Comparative ultrastructure of rotifer vitellaria

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The vitellarium or yolk gland of rotifers is a large syncytial organ that functions to synthesize nutrients, eggshell precursors, and provide cellular organelles to developing oocytes. Oocytes in the germarium receive these products via cytoplasmic bridges, making the

germovitellarium the largest syncytial organ in the body. Despite its size, there is limited data on the ultrastructure of the germovitellarium and the synthetic products it shuttles to oocytes. Here, we provide details on the vitellarium of 20 species across Monogononta including species of *Acyclus, Brachionus, Collotheca, Epiphanes, Euchlanis, Floscularia, Hexathra, Kelicottia, Keratella, Limnias, Platyias, Polyarthra, Pompholyx, Scaridium, Sinantherina, Stephanoceros, Synchaeta*, and *Trichocerca*. Our results reveal the vitellaria to be relatively similar across species with respect to being multinucleated, syncytial, synthetically active, and ensheathed in a follicular layer. Differences are mostly observed in the size and types of secretion granules produced by the vitellaria. Most species we examined were wild caught and so differences in age, diet, reproductive status and environmental conditions may account for some of these differences. We outline future directions for this research and provide important insights into why studies of vitellaria may help to understand potential correlations between resource availability and phenology.

The retrocerebral organ (RCO) of *Trichocerca similis* with a review of the RCO in other Rotifera

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The retrocerebral organ (RCO) of rotifers is hypothesized to be an exocrine gland that secretes mucus to the apical field of the corona. The secretions presumably aid in locomotion of benthic rotifers but may also function to help attach eggs to a surface. While the RCO is widely distributed across Rotifera, its ultrastructure is mostly unknown. Here, we use TEM to inspect the RCO of *Trichocerca similis*, a planktonic rotifer with an RCO that is composed of a subcerebral gland, a reservoir, and a single duct that traverses the cerebral ganglion. Results reveal the subcerebral gland to be binucleate and with a cytoplasm containing abundant mitochondria, rough endoplasmic reticulum, Golgi, and secretory granules. Electron-dense, immature secretion granules accumulate at the apical end of the gland and undergo homotypic fusion to create larger granules with mesh-like contents. Mature secretion granules combine into larger globule-shaped secretions that are exocytosed to the reservoir lumen, eventually taking on a long, spindle shape. During exocytosis, the secretions appear to lose their plasma membranes, probably through a membrane-recycling pathway. The secretions maintain their shapes in the reservoir because each is bound by electron-dense filaments that replace the plasma membrane. The secretions become tightly coiled within the reservoir prior to extrusion through an anterior duct. Secretion ultrastructure suggests they are likely to be hydrated glycoproteins. We also present a review of the RCOs across the Rotifera to compare what is known about their structure, functions, chemistries, and potential homologies.

Microplastics ingestion and egestion in the predatory rotifer *Asplanchna brigthwelli*

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Recently, microplastics have been associated with different demographic alterations in freshwater zooplankton and have also been recorded in more than 250 species of fish linked to human consumption, so this kind of pollution should be studied urgently to clarify the processes occurring in the aquatic food web. In this study, we evaluated the effect of two microplastics (1 and 10 µm) in the ingestion-egestion processes on the predatory rotifer *Asplanchna brigthwellii*. We fed the predatory rotifer with prey (*Brachionus caudatus*) exposed to fluorescent polystyrene microplastics (0.1 µg ml/L), for this purpose the predators were previously subjected to 4 h of starvation and then fed with the treated prey for 2 h. After the feeding, 10 asplanchnids were individually placed in clean media with prey (1 ind. ml/L) without microplastics to keep them active for egestion evaluation. At six time points (0, 4, 8, 24, 48, and 72 h), asplanchnids were randomly collected, fixed with 4% formaldehyde and observed under a fluorescence microscope. Our results indicate a gradual decrease in fluorescence over time with relative fluorescence values between 190 to <150, and, however, microplastics were visible up to 48h after exposure. Processes such as bioaccumulation and biomagnification of this type of pollutants could have important impacts on different trophic levels.

Salinity effect on the rotifer community structure of a shallow eutrophic lake: A laboratory study

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Lake Xochimilco (Mexico City, Mexico) is a eutrophic shallow lake. The lake has been receiving partly treated wastewater and fertilizers from surrounding agricultural fields, during the last several decades. Although salinity levels have not exceeded 0.5 mg/L, there is the possibility that salinity levels may increase as a result of climate change with consequences on the zooplankton composition, especially rotifers, which dominate in this waterbody. In order to test the effect of different concentrations of salt (NaCl) on the rotifer composition, we filtered lake water using 200 µm mesh size and removed all larger organisms. The filtered water containing mainly rotifers and copepods was distributed into a total of 12 test jars of 3 L volume. Each treatment contained one of the four salt concentrations (0, 0.25, 0.5 and 1 g/L). For each treatment we maintained 3 replicates. Following initiation of zooplankton exposure to different salt levels, after every 3 days, we sampled 350 ml of water from each jar and preserved the zooplankton community in 4% formalin. The

experiment was terminated after 3-4 weeks. We found significant differences between controls and salinity treatments. The densities of *Brachionus calyciflorus*, *Keratella cochlearis* and *Polyarthra vulgaris* decreased with increasing salinity level and exposure time, while two other species *Brachionus angularis* and *Brachionus budapestinensis* were nearly eliminated.

Impact of different sources of dissolved organic carbon on rotifers in shallow lakes

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Climate changes cause increased input of dissolved organic carbon (DOC) in shallow lake ecosystems, leading to alterations in physico-chemical factors, composition of zooplankton and food web interactions. Rotifers, as an important component of freshwater plankton, significantly contribute to the carbon transfer from lower to higher trophic levels. In this study *in situ* mesocosms were used to determine the impact of labile and recalcitrant carbon on lake ecosystems. The main objectives of this study were to assess: (i) structural and functional traits of rotifers; (ii) the influence of abiotic and biotic factors on composition of rotifers; and (iii) ecosystem recovery after impact of different sources of DOC. Higher diversity, abundance and biomass of rotifers were observed in the mesocosm with labile carbon compared to the mesocosm with recalcitrant carbon. During the experiment, planktonic nanoalgivor rotifer species (*Keratella cochlearis*), dominant at the beginning, were replaced by semiplanktonic detritivore species (*Lecane, Lepadella*) dominant at the end of the experiment with labile and mixed DOC. That shift in the rotifer assemblage was likely caused, first, by higher concentrations of chlorophyll *a* as well as presumably competition with crustacean algivores at the beginning, and second, higher concentrations of DOC and nutrients at the end of the experiment. The results of this study indicate the possibility of ecosystem recovery after external DOC load, replacement of species which lead to assemblage more adopted to the stressor impact, and the importance of rotifers as indicators in assessing changes on the ecological status of shallow water bodies.

Zooplankton in the two lagoons of different salinity and trophic state (Turkey)

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Coastal lagoons are the most important wetlands in terms of biodiversity. The study was conducted seasonally between 2014 and 2016 years in Dalyan Lagoon in Kocaçay Basin, which is mesohaline ecosystem (between 7-11 ‰) and located in the Southern Marmara region, and Gici Lagoon in Kizilirmak Basin which is

oligohaline ecosystem (between 0,3-0,7 ‰), located in the North Anatolia region of Turkey. In this comparative study, it was attempted to assess impact of trophic state on zooplankton assemblage in lagoon lakes from different geographical regions. A total of 45 zooplankton species were identified in Lagoon Gici of higher trophic state (38 species belong to Rotifera; 5 Cladocera and 2 Copepoda) while 19 zooplankton taxa were identified in Dalyan Lagoon of lower trophic state (15 species belong to Rotifera, 4 species to Copepoda). In the mesohaline Gici Lagoon, Rotifera made up 90%, and cladocerans and copepods only 10% of zooplankton. Also, rotifers were represented for the longest time period and the most individuals belong to the *Brachionus* genus. However, in oligohaline Dalyan Lagoon, zooplankton assemblage was dominated by copepodites and nauplii (86%), with much smaller share of Rotifera (11%), and Copepoda (3%). Variations in diversity were observed due to changes in salinity and temperature parameters depending on seasonal changes. Furthermore, it is concluded that salinity and climatic differences between the lagoons have created significant differences in the composition, but no pattern was detected in the plankton distribution between the two lagoons.

Rotifer´s assemblage and seasonal distribution in two shallow lagoons in Turkey

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In this study, temporal variations in abundance, biomass and size structure of rotifers were determined and compared in the samples between 2014 and 2016 years for two shallow lagoons located in different climate zones. Paradeniz Lagoon, is high saline ecosystem (around 31‰), located in the Mediterranean Sea Region, on the south coast of Turkey and formed within delta of the Göksu River. Uzungöl Lagoon is low saline ecosystem (≤ 1‰) located in Kızılırmak Delta, in north of Turkey. Key environmental parameters, particularly temperature, salinity, conductivity, dissolved oxygen, and chlorophyll a, were determined in interactions with rotifer´s assemblage. Rotifera were the dominant group in the zooplankton of slaine, Uzun Lagoon, especially species *Keratella cochlearis, K. quadrata, Polyarthra vulgaris, Filinia longiseta, Notholca acuminata* and *Brachionus calyciflorus* were made up more than 70% of the rotifer community. In slightly saline Paradeniz Lagoon rotifers *Synchaeta pectinata* and *Hexarthra fennica* were constant species but their population density was very low, whilst copepods were made up more than 90% of the total zooplankton. Moreover, an increase nauplius lengths correlated with maximum salinity in both lagoons. Results of this research indicated that climate-dependent salinisation of shallow lagoons is an important factor for ecological explanations of biodiversity, density and biomass of the zooplankton.

***Syncheta*'s community in the urban sea front of the Thessaloniki Bay**

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Among marine zooplankton, rotifers' presence and significance is being underestimated, mainly due to smaller size fractions lost through the large mesh size nets commonly used in marine zooplankton studies. In addition, samples' preservation, often makes species identification difficult, explaining why their role in coastal ecosystems has not been fully determined. The aim of the present study is to investigate the temporal distribution of the rotifer's genus *Synchaeta* in the marine environment of the Thessaloniki Bay. Samples of microzooplankton, collected using a 50 μm mesh size plankton net, were conducted on a weekly basis, within two sampling periods in 2020 and 2021. Four species of the genus *Synchaeta* (*S. baltica*, *S. grimpei*, *S. neapolitana*, *S. vorax*) were identified, with *S. baltica* and *S. neapolitana* being the most abundant. Salinity showed a significant positive impact on *S. baltica*'s variation in abundance in both sampling periods. In 2021, pH was the determining variable, influencing *S. baltica* and *S. neapolitana* distribution, negative and positive, respectively. Results also indicate coexistence patterns among all *Synchaeta*'s species, as well as competitive trophic relationships with other filter-feeding taxa, as tunicate *Oikopleura* sp. and cladoceran *Penillia avirostris.* The ongoing samplings in the study area, in order to complete an annual cycle, will provide a holistic understanding of *Synchaeta*'s role among the taxonomical and functional components of the Thessaloniki's Bay planktonic food web.

Oxbows as a habitat of high diversity of Rotifera and Crustacea communities in small water bodies

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In order to analyse the influence of the origin of small water bodies on the diversity and structure of rotifers and crustaceans, a group of almost 300 ponds (natural post-glacial, oxbows and artificial - clay pits, gravel pits, turf-excavation pits) was compared. The ponds were located in different types of catchment (forest, agricultural, urban) in central-western Poland. Sites located in the open water area and among macrophytes were considered. In total, 389 zooplankton taxa (286 Rotifera, 103 Crustacea) were identified, with oxbow lakes (306 species in total) showing the highest species richness, the greatest share of species considered rare in Poland and exclusive species of high frequency (e.g. *Trichotria pocillum, Lepadella triptera, Scaridium longicaudum, Ascomorpha ecaudis, Lepadella rhomboides, Squatinella rostrum, Keratella ticinensis*), in comparison to artificial (283) and glacial (279) ponds. Zooplankton diversity is usually related to spatial and temporal variability of the environment. However, a pond's origin, which may affect the overall functioning of the biocoenoses, and thus the specificity of organisms, also significantly differentiates zooplankton. Oxbows, of best environmental conditions (the lowest conductivity, DIN chlorophyll concentration and the highest transparency), had the most diverse zooplankton, which was largely related to their location within protected areas with a

low degree of human transformations (Natura 2000 areas, landscape parks). That is why oxbows, as an important element of river valleys, are valuable elements of the water landscape.

Taxonomic approach as a sensitive tool to assess distinctiveness between the two temperate climate European regions, northern (Poland) and southern (Croatia)

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Rotifer structure may serve as predictor of environmental conditions, including long-scale distance between shallow water bodies. As functional traits were not a sensitive tool to assess latitudinal variation in the northern-southern temperate European regions during spring and autumn seasons, we assumed that a taxonomic approach, referring to certain dominating species, will be a better method. An analysis of abiotic features showed a high similarity concerning water bodies in Poland and Croatia in the spring, while a clear latitudinal segregation was obtained for the autumn season. This is due to the variation of trophic conditions, with a much higher trophic state, reflected in the increases in chlorophyll a concentration and phosphates, in the case of Polish ponds in the autumn period. 17 rotifer taxa dominated, with only 7 dominating in both countries (Bdelloidea, *Brachionus angularis, Filinia longiseta, Keratella cochlearis, Keratella quadrata, Polyarthra* ssp., *Synchaeta pectinata*), however, only *K. cochlearis* and *Polyarthra* ssp., dominated in each country in the spring and autumn. There was a group of species that dominated exclusively in Croatia (*Colurella uncinata, Gastropus stylifer, Lepadella patella, Trichocerca porcellus*) or only in Poland (*B. quadridentatus, Cephalodella catellina, Eosphora ehrenbergi, K. cochlearis tecta, Notholca acuminate, N. squamula, Synchaeta lakowitziana*). *K. cochlearis* had the highest frequency, dominating in over 60% of samples in Croatia. A distinctiveness between the two European regions, northern and southern, with temperate climates, was obtained at the level of rotifer taxa. Thus we recommend a taxonomic approach for latitudinal studies in a seasonal aspect.

Characteristics of rotifer community structure and species diversity in the typical river- lake interconnected ecosystem

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Urban river-lake interconnected ecosystems are formed by the artificial connection between lentic and lotic water bodies. During 2017-2019, surveys were conducted in the two interconnected ecosystems in Changde city and Guangzhou city, China. We recorded 95 and 100 rotifer species in the ecosystems in Changde and Guangzhou, respectively. In Changde city, the highest number of taxa

occurred in Lake Liuye (87), followed by the connecting waterways (58), and lowest (31) in the Yuan River. Altogether 22 taxa were shared among the three water bodies in Changde. In Guangzhou city, the highest number of taxa occurred in Guangzhou Segment of Pearl River (85), followed by the connecting waterways (68), and the lowest in Lake Haizhu (56) in Guangzhou. *Polyarthra dolichoptera* was the dominant species in Changde, while *Polyarthra vulgaris* dominated in Guangzhou. Rotifer abundance ranged from 10 to 2628 ind./L. The average abundance in the Yuan River was significantly lower than that in Lake Liuye. In Guangzhou city, the average abundance in Guangzhou Segment of Pearl River was significantly higher than that in Lake Haizhu. Temperature and trophic state were the key factors for spatial-temporal variation of rotifer community in the two urban river-lake ecosystems. Rotifer species richness and abundance showed positive correlation with the trophic state. This study suggested that the rotifers indices is a reliable indicator of water quality in urban river-lake ecosystems in South China. The heterogeneity of the water environment provides diverse habitats for sustaining rotifer diversity.

Rotifers and trophic state at high altitude tropical shallow lakes

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Rotifer community structure was studied in the four, small, adjacent and interconnected shallow lakes (Z<1.5 m) which located at high altitude (2300m a.s.l.) in the south area (La Cantera Oriente) of Mexico City. Monthly samplig was carried out for a year. Through clorophyll a and the Secchi disc transparency the Carlson Trophic Index (TSI) was calculated and the results were as follows: Spring 28-46, oligo-mesotrophic; Regulation Lake: 34-53. Meso-eutrophic; North Lake: 58-75 light eutrophic; Central Lake: 60-80, eutrophic; South Lake: strongly eutrophic. Forty rotifer species were identified and the dominant species were *Keratella cochlearis, Polyarthra vulgaris*, and *Synchaeta oblonga*. Twenty three species were found in eutrophic conditions and 35 in the oligo-mesotrophic ones. The highest value of similarity (Jaccard 0.74) was recorded in the two least productive water bodies, and the lowest (0.48) in the eutrophic and oligo-mesotrophic. When using abundances (ANOSIM) the only water bodies with no difference (R=0.043 p=0.97) were the North and the Center lakes. *K. cochleris* was the species that contributed the most (SIMPER 34%) to the difference between the water bodies, when using abundance data, while *P. vulgaris* was when biomass was considered. Rotifers composition, abundance and biomass adequately reflected the differences in trophic status in the strongly interrelated water bodies.

Effects of the environmental endocrine-disrupting chemicals triclosan on life-cycle and population parameters of rotifer *Brachionus plicatilis*

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Triclosan (TCS), typically environmental endocrine-disrupting chemicals (EDCs), was widely distributed in the water environment. TCS could spread through the food chain and possess a risk to aquatic animals. In this study, the effects of five different concentrations of TCS (0 μg/L, 25 μg/L, 50 μg/L, 100 μg/L, 200 μg/L) on the life-cycle parameters and population parameters of rotifer *Brachionus plicatilis* were investigated. Results showed that the life-cycle parameters were significantly affected by TCS. Exposed to 25 μg/L TCS, the number of total offspring per female increased significantly, while the number of total offspring per female decreased significantly exposed to 200 μg/L TCS. The lifespan and the reproductive period of rotifers were significantly shortened at high concentrations of TCS (200 μg/L). In the population experiment, the population density and population growth rate were significantly reduced by 200 μg/L TCS. The results of the net reproductive rate (R0) increased significantly at the low concentrations (25 μg/L) of TCS. However, R0 and generation time (T) decreased significantly at high concentrations of TCS (100 μg/L and 200 μg/L). These results suggested that the life- cycle parameters and population parameters of rotifers were vulnerable to TCS exposure, and rotifers had a great potential to be used for minoring and assessing TCS pollution in the water environment.

Reproductive isolation in clones of the marine rotifer *Brachionus* cf. *ibericus* "Quintana Roo" México strain

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In the Yucatan peninsula, there is no reproductive and morphometric analyses of clones of the *Brachionus plicatilis* species complex: a group of great economic, ecological, and evolutionary importance. Recent studies have identified 15 different genetic lineages around the world. Therefore, our main objective was characterizing clones of this group to generate baseline information for the Yucatan peninsula, specifically in the state of Quintana Roo. For this rotifer cultures were established. Collections were carried out in two study areas in the southeast and northwest of Quintana Roo. The collections were made with a Wisconsin-type zooplankton net with 54 μm mesh size. From fresh zooplankton samples individuals from the *Brachionus plicatilis* species complex were isolated and cultures were established in a bioclimatic chamber at 25°C with a photoperiod of 12:12 hours of light and darkness. Rotifers were fed with 1×10^6 cells/mL of the microalgae *Nanochloropsis oculata*. Subsequently, after two months of adaptation to laboratory conditions, we: a) performed morphometric characterization, b) established monoclonal cultures, c) estimated population growth, d) hatching percentage of resting eggs, and e) performed cross-mating experiments. Twelve clones were obtained from northwest and eleven clones for southeast Quintana Roo. Three sizes corresponding to medium morphotype (SM) of the species complex were identified in each locality. We found differences (between areas and the different sizes) in the production of females, males, and resting eggs, as well as in their percentage of resting egg hatching. Reproductive isolation was determined,

suggesting two areas of speciation in Quintana Roo: southeast and northwest.

Chihuahuan Desert rock pool rotifer assemblages

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Rock pools are small and structurally simple systems, and they serve as model systems for investigating the influence of environmental factors on community assemblages. Other than our work in the Chihuahuan Desert, there has been little research on temporary rock pool communities of North America, and most did not include one of the most dominant groups; the Rotifera. We predicted rotifer species richness would increase with longer hydroperiods and at lower latitudes. Richness was assessed from rock pools in six locations within a latitudinal gradient of 26°N - 34°N. At least 3 sets of 3 rock pools were sampled from each location. Rotifers were observed in 77% of the 62 rock pools. A total of 13 families, 19 genera, and 28 species were identified and bdelloids were the most commonly found taxon. Rock pools were categorized into four hydroperiod levels for the analyses. As predicted, species richness was positively correlated ($R2=0.29$; $p < 0.05$) with hydroperiod. There was no correlation of species richness and latitude. Analysis of Sorenson's diversity index revealed higher community dissimilarity at local scales ($\beta = 0.78$) than regional scales ($\beta = 0.65$). These findings are consistent with previous results showing that rotifer richness is higher in pools with longer hydroperiods, and diversity is homogenized over larger geographic scales. Newly sampled sites contained several taxa not previously reported, indicating that additional sampling sites improve estimation of species richness. Our results could provide guidance for conservation prioritization of Chihuahuan Desert freshwater habitats and establish ecological baselines for temporary environments.

Status update on defining the *Brachionus plicatilis* cryptic species complex

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We propose a new organization for three lineages in the *Brachionus plicatilis* cryptic species complex: the *Brachionus plicatilis*, *Brachionus ibericus*, and *Brachionus rotundiformis* cryptic species complexes. These lineages are easily identified by the morphology of the gastric glands and the presence or absence of stippling on the lorica. Because this split is supported by morphology and reproductive data, we posit that these three lineages are distinct and genetically isolated. Based upon all available COI sequences uncorrected intraspecific divergences for new species of the *Brachionus ibericus* cryptic species complex are as high as 7.92%, with interspecific values ranging from 12.82%

to 19.71%. This is corroborated by ITS1 findings with intraspecific variation as high as 2.2% and interspecific variation ranging from 4.16 to 10.07%. In presenting evidence for these lineages, we also assign species names to six members of the *Brachionus ibericus* cryptic species complex.

Which environmental parameters shape the zooplankton asemblage in canals?

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In our study we investigated how the physico-chemical and biological parameters shape zooplankton community in artificial waterways. Samples were gathered monthly during growing season 2019 from the Bydgoszcz Canal, the Noteć Canal and the Brda River (Poland; Europe). The studied Bydgoszcz Canal is an important link between the catchments of two large European rivers (Vistula and Oder). During the research, we noted a significant influence of some physico-chemical parameters on the structure of the zooplankton community. The primary production variables (oxygen concentration, water pH and chl-*a* concentrations) shaped the zooplankton community, especially density and biomass of rotifers in the studied canals. Rotifers dominated qualitatively and quantitatively among the zooplankton at canals. In total, we recorded 98 species of zooplankton. Rotifers constituted 73 and crustaceans 25 species. The average density of zooplankton was 320 ind/L. The average density of rotifers was 259 ind/L, which accounted for over 80% of total zooplankton density. The average biomass of zooplankton was 1.89 mg/L and crustaceans constitute more than 88% of total zooplankton biomass. Due to the lowest water flow in the Bydgoszcz Canal compare to Noteć Canal and Brda River, the highest abundance and biomass of zooplankton, including Rotifera, were observed there. It is likely that locks, reduce water flow in the canals, create better conditions for macrophyte growth. Macrophytes form ecological niches that favor zooplankton development.

Are rotifers good indicators of reference lakes?

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The Water Framework Directive (WFD, 2000) requires the assessment of the ecological status of water bodies to express a deviation of the current status from the reference conditions. Despite fact that the zooplankton is an integrative biological component of pelagic ecosystems, this group of organisms, has not been included in the WFD as a biological quality element required in lake ecological status assessment. In zooplankton community Rotifera well reflect trophic state because they respond directly to eutrophication pressure due to being independent of fish predation. I attempted to explore whether Rotifera can be also a good indicator of reference conditions. The aim of the study

was to describe the characteristic of Rotifera community composition for Polish lakes that are in near-pristine conditions and largely unaffected by anthropogenic pressures (reference lakes). The study was carried out in 2012-2015. Using the SIMPER analysis and Indicator Value the characteristics of Rotifera community in un-impacted lakes were defined. The Indicator Value analysis listed 3 typical reference Rotifera taxa that include: *Ascomorpha ecaudis*, *Collotheca pelagica*, *Gastropus stylifer*. *Pompholyx sulcata* was associated with non-reference conditions with the highest Indicator Value. Presented results provide one more evidence that zooplankton is a good and valuable indicator of ecological status and should be included as one of the Biological Quality Element for the WFD.

Cyanobacteria defense against ciliate grazers - not so easy in the presence of *Lecane* rotifers

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Cyanobacteria can apply different modes of defense against their enemies. They can react to the presence of grazers „on demand" in a form of inducible defense. The examples of such defense are ability to form dense clumps of mat and to accelerate the production of protective sheaths and mucilage in the presence of a ciliate grazers. As it was shown that rotifers can feed on biofilms, we tested if rotifers, by grazing on exopolysaccharides, weaken the defense of cyanobacterium against ciliate grazer. The experiment was conducted in culture test plates inoculated with small piece of cyanobacterial mat of *Phormidium* (Ph2). Ciliates *Furgasonia blochmanni* (F) and rotifers *Lecane inermis* (L) were transferred into wells to attain the following experimental setup: F+Ph2; F+L+Ph2; L+Ph2 and Ph2. To reflect the range of mat's compactness we used a value of mean autofluorescent brightness of the initial piece of mat. The higher the brightness, the higher compactness. Every day the percentage of satiated ciliates was calculated. At the end all ciliates, cysts, and rotifers were counted. In the presence of ciliates alone cyanobacterium defended itself effectively by hiding trichomes endings inside dense clumps covered with mucilage. The presence of rotifers feeding on cyanobacterial mucilage led to decreased effectiveness of defense in two ways: by increasing dispersion of cyanobacteria and loosening cyanobacterial mat and by ingestion of exopolysaccharides covering trichomes. In the presence of rotifers and ciliates, almost all trichomes were removed. It is the first report showing how rotifers can weaken the defense of cyanobacteria.

Trapped with a foe: genomic insights into the evolution of a bdelloid rotifer and its fungal pathogen co-cultured on a dish for over five years

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Red Queen-type arms races are posited to

play an important role in the generation of biodiversity, driving evolutionary novelty and speciation in coevolving host-pathogen or predator-prey clades. Conversely, the inability to 'keep up' with coevolving parasites is often invoked as a cause of early extinction of asexual lineages. Here we present the results of an unreplicated experiment in which a lineage of the bdelloid *Habrotrocha elusa* was co-cultured with a weakly pathogenic fungus *Rotiferophthora brevipes* on a single Petri dish. Thanks to algal contamination of the culture, the bdelloid could coexist with its parasite for over five years in a self-sustaining mesocosm, with no external input besides deionised water. I will report on genome assemblies for *H. elusa* and *R. brevipes* isolates sampled both before and after the co-culturing period. Specifically, I will discuss mutations in the co-cultured genomes relative to their 'naive' ancestors, with a particular focus on genes potentially involved in defence (*H. elusa*) and pathogenicity (*R. brevipes*). This analysis offers insights into the short- term evolution in an apparently clonal host-pathogen system, to complement investigations of the longer-term coevolutionary history of bdelloids and their fungal pathogens.

Peatland paleoenvironmental changes are reflected in the rotifer community since the Late Glacial: A case study of the raised bog and peatland in Poland

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In paleolimnological studies rotifers are a very rare component in multiproxy research. These small invertebrates are identified from sediments by their loricas. Analysis of subfossil rotifers was conducted for sediment cores collected from two peatlands: the Imszar raised bog (NE Poland, Podlasie Region) and from the Podemszczyzna peatland (SE Poland, Sandomierz Basin). Results were compared with multiproxy analyses of peat deposits: pollen of aquatic and mire plants, non-pollen palynomorphs (NPPs) and geochemical analyses. Analyses of rotifersindicated two species: one bdelloid, *Habrotrocha angusticolis* and one monogononta, *Keratella cochlearis*. In both profiles, the occurrence of rotifers was not correlated with pollen of aquatic or mire plants or NPPs; they seem to be more related to geochemical indices. Highest densities of rotifers were found in profile sections without significant fluctuations of geochemical variables, i.e., those with higher Na/K (chemical denudation) ratios and stable redox conditions (Fe/Mn ratio). The study of the subfossil rotifers supported by multiproxy analysis of peat deposits could be a useful tool for indication of periods with stable paleohydrological conditions during the Late Glacial and Holocene.

Joint effect of high temperature and low oxygen level on body size of rotifer *Lecane inermis*

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The global increase in ambient temperature is a threat for living organisms, especially ectotherms. They are equipped in several mechanisms to counteract the negative consequences of warming. Among them is the temperature–size rule (TSR), a phenomenon which manifests as phenotypic decrease of body size with increasing temperature. It is predicted that such a response is mechanism of counteracting the reduced efficiency of oxygen delivery to cells, because oxygen availability naturally decreases with increasing temperature. We hypothesized that body size of ectotherms would be smaller at higher temperature and that this effect will be enhanced after exposition to two stressors simultaneously – high temperature and low oxygen level. In our study, four clones of rotifers *Lecane inermis* experienced three levels of temperature, 25°C, 30°C, and 35°C, under normoxia and hypoxia. The body size of rotifers was measured after two and four days of exposition to experimental conditions. The effects of temperature and oxygen were clone-specific, which means that either the former or the latter parameter was more important in driving body size of specific clone. Moreover, the pattern changed in time, and, depending on the clone, the effect of both parameters was either enhanced in comparison to earlier measurements, or it was considerably different. We suggest that different thermal preferences resulting from previous experience of each clone have important effect on the clonal strategy of dealing with stressful thermo-oxygenic conditions. Such subtle differences in closely related organisms show how complex the overall pattern in community response to environmental changes may be.

The combined effect of microplastics and temperature on competition between *Brachionus havanaensis* and *Brachionus calyciflorus*

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Because of the mass production, the residues of plastics are very common in water bodies. The aquatic organisms are frequently exposed to wastewaters, industrial and domestic effluents with microplastics from clothes or personal care products. The effects of microplastics in aquatic organisms can be diverse, from mechanical damage by ingestion to intoxication associated to chemicals adsorbed by microplastics. The presence of microplastics and the temperature variations are strong stressors to aquatic biota and their interactions. Rotifers as *Brachionus havanaensis* and *Brachionus*

calyciflorus are common in freshwaters, these organisms are filter feeders nonselective capable to consume microplastics particles between 0.05 to 50 µm that can affect the demography and the interactions between species. Here, we evaluated the effect of 30 µm beads of polystyrene microplastics (SIGMA-ALDRICH) at 10 and 20 mg/L on population growth and competition of *B. havanaensis* and *B. calyciflorus* using 0.5 x 106 cells de Chlorella vulgaris as food at 20 and 25º C. There was a decrease in the population growth in treatments with algae and presence of microplastics at both experimental temperatures when compared to the control group in both species cultured alone, and an increased the survivor of *B. calicyflorus* in the competition experiments. Treatments with only microplastics showed a decrease of the population before the 5th day.

Motility and size of rotifers as two risk factors for being consumed by the passive protistan predator *Actinosphaerium* sp.

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Predation is a well-known factor that structures rotifer communities. However little attention has been given to the role of protists as predators. Here, the predatory behavior of *Actinosphaerium* sp., a freshwater heliozoan, on 7 rotifer species was investigated. Predators and prey were collected from a local playa; with the exception of *Brachionus calyciflorus* (commercial laboratory stock) that served as a naive prey control. Prey included large species (>300 µm max. length: *Asplanchna sieboldi, B. calyciflorus, Cephalodella gibba*) and small species (<300 µm: *Euchlanis dilatata, Lepadella patella, Platyias quadricornis* and *Lacinularia flosculosa*). Three experiments were conducted. (1) Single prey items offered for 40 min in 1 ml of media. Large prey were ~1.7 to 3.0 times more likely to be ingested than small prey. No *L. flosculosa* were ingested, even after 72 hrs, contrary to field observations. (2) Preference tests with all prey items. Ivlev's electivity index showed *A. sieboldi* (0.33) and *B. calyciflorus* (0.33) as favored prey items. Low or negative values were found for *C. gibba* (0.20), *P. quadricornis* (-0.33), *E. dilatata* (-1.00), and *L. patella* (-1.00). (3) Growth rate of *Actinosphaerium* sp. on mixed prey diets with and without *Asplanchna*. Tests were monitored after 24, 48, and 72 hrs. The highest growth of *Actinosphaerium* sp. was observed in the presence of *A. sieboldi*. In addition, no correlation between reported swimming speeds and ingestion was found. This study suggests that motility and size are two risk factors that influence rotifer predation risk by passive protist predators.

Rotifer community composition along the increasing salinity gradient - a pilot-study in a brackish marsh (Palud, Croatia)

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Palud is a natural depression in the southwest of the Istrian peninsula (N Adriatic Sea, Croatia), situated by the sea and transformed into a marsh by the inflow of water from the adjacent hills. At the beginning of the 20th century, the marsh was connected to the sea by an artificially dug channel to control mosquitoes (vectors of malaria). This led to the brackish conditions in the marsh. The objective of this pilot-study was to investigate the rotifer community composition in Palud Marsh during the summer of 2021. Zooplankton samples were collected at two-week intervals during July 2021 at the four sampling sites that extend along the Palud salinity gradient. Water physicochemical factors analysis showed a significant trend along the salinity gradient indicating a decrease in plankton productivity in July, likely caused by a decrease in Palud water depth due to runoff from the marsh to the sea. Only a very small number of taxa (6) were found in the plankton. Rotifers were represented by three taxa (*Brachionus plicatilis*, *Encentrum* sp., *Lecane lunaris*). Of all individuals recorded, 97.3% belonged to *B. plicatilis*, a cosmopolitan eurythermal species and a halobiont (i.e., it prefers saline shallow waters and lagoons). This pilot-study was the first step in assessing the ecological conditions of Palud Marsh. Further studies are necessary to develop effective conservation measures for this protected habitat, which was declared a special ornithological reserve in 2001.

Hotel Teasel, the multi-story temporary accommodation for rotifers

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Phytotelmata and their analogs (e.g., pitcher plans, tank-bromeliads, discarded cups) are useful tools in the study of several important ecological processes, including dispersal, colonization, and anhydrobiosis in response to desiccation. As the model plant for this study, we chose teasel (*Dipsacus fullonum*) a biennial plant that is the most common phytotelmata species in Slovakia. The rotifer communities inhabiting phytotelmata can be adopted for the investigation of ecological processes for several reasons; they are small, numerous, amenable to experimentation, exist in various degrees of isolation, and are relatively simple systems to study. Nevertheless, they are important components of the ecology of Slovakia. When mature teasels, have, on average, 8 levels of rosettes (floors) with a water volume of ~130 ml per axil. From axils of 30 plants, we identified 5 rotifer taxa. Bdelloids were the most frequent group that we recorded (3244 ind./L); they represented ~92% of all rotifer specimens. *Habrotrocha* and *Philodina* were the most frequently encountered genera; these were followed by lecanids (*Lecane inermis* 175 ind./L and *Lecane bulla* 112 ind./L) and *Colurella obtusa* (8 ind./L). Beside live samples, we also collected dried sediment from axils at the end of winter. In the second part of this study, we plan to test the recovery efficiency of different rotifer taxa after varying periods of anhydrobiosis.

A meta-synthesis of the biogeographical distribution of Rotifera in Africa

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The rotifer fauna of Africa has been studied for >100 years, but there has been no recent synthesis. We compiled data from 265 publications that reported information on African rotifers. Our dataset yielded information on the distribution of 770 taxa from ~1850 separate habitats; these included both natural and artificial habitats such as lakes, paddies, ponds, puddles, oases, outdoor mesocosms, reservoirs, rivers, water treatment ponds, and wetlands. A heat map of predicted rotifer biodiversity indicated that the greatest diversity should be present in the sub-Saharan region including a large hotspot in Mali and several smaller ones scattered throughout other states in that region. Lakes Kariba, Tanganyika, and Malawi also showed high diversity, but surprisingly, Lake Victoria had lower diversity than expected. Two regions showed unusually high predicted diversity: northwestern Algeria extending into Morocco and Egypt concentrated along the Nile River. Equatorial Africa is rich in habitats well suited for rotifers, yet their predicted biodiversity seems low. Latitude and elevation were negatively correlated with richness, while permanent water source and littoral zone were positively correlated according to Generalized Linear Modeling results. Partial RDA analyses showed significant correlations among environmental features and species occurrences, including the habitat types: lake, savannah, and anthropogenic. Joint Species Distribution Modeling showed two groups with positive associations: predominantly planktonic species and a suite of littoral species. It is clear from our review, that more survey work remains to be done to achieve a better understanding of the distribution and ecology of African rotifers.

A meta-analysis of LC50 values in Rotifera

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Lethal concentration (e.g., LC50) is an essential indicator for the assessment of acute toxicity of chemical compounds and elements, such as heavy metals (and metalloids). In rotifers, a considerable number of LC50 tests have been conducted for metals under diverse experimental conditions. However, the significance of tested variables (e.g., species, oxidation state, and exposure time) in LC50 values has not been determined. Accordingly, we compiled all rotifer LC50 metal and metalloid data, and conducted a meta-analysis that will allow researchers to 1) identify sensitive and tolerant taxa, and 2) determine significant factors that affect their tolerances. We found 233 LC50 values for 13 metals (Ag, Al, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Sn, and Zn) in 19 species. LC50 values varied within and between species: Cd values were the most variable (LC50 14.8 to 56,000 ppb) and together with Cu (LC50 3.7 to 1,900 ppb),

were the most investigated metals. *Brachionus calyciflorus* was the most tested species (26 LC50 values for 11 elements); *Philodina acuticornis* and *B. plicatilis* were most tolerant to Cu and Cd, respectively, while *Euchlanis dilatata* was the most sensitive species to both metals. To understand differences in LC50 values, we will discuss the conditions and methods under which LC50 values were determined, as well as study possible patterns related to phylogeny and sensitivity to provide further information that facilitates the prediction of rotifer responses to metals and inform experimental design for future research. The database will be made publicly available upon publication.

Rotifer species richness along the altitude gradient of glacial lakes at the Adriatic basin (Albania)

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Eight Glacial Lakes within Albanian part of Adriatic Sea basin were subject of rotifer fauna survey in the period of 2018-2021. All of the lakes are located at altitudes above 1610 m except Lake of Dushku (1380 m). A total of 31 rotifer taxa were identified. Some of the identified taxa are new records for the Albanian inland water fauna and they include: *Lecane latissima, Cephalodella gibba, Notholca squamula* and *Euchlanis dilatata lucksiana*. Among the species the most dominant one were *Keratella cochlearis* (7 lakes), *Lecane lunaris* (7 lakes) *Keratella quadrata* (6 lakes), *Brachionus quadridentatus* (4 lakes*), Notholca squamula* (4 lakes), *Filinia longiseta* (4 lakes) and *Lepadella patella* (4 lakes). Following revealed data there is a linkage among rotifer species richness along different altitudinal distributions of lakes, where Lake of Dushko (1380 m) is distinguished by 16 taxa, while Lake of Valamare (2070 m) with four taxa. There is undergoing eutrophication process accelerated by cumulative anthropogenic impact including deforestation, fire events, erosion and siltation, where the most affected one is Lakae Dragani where 4.3 mg/l dissolved oxygen and PO_4^{3-} - P (µg/L) was at rate of 9.03. The current survey enforces the recognition of ecological and social importance of continental small water bodies and further on it appeals for the conservation needs these aquatic ecosystems.

Rotifers community in a newly established reservoir during the impoundment of a hydropower dam

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The global increase in energy demand has triggered a global bloom in the construction of hydropower dams worldwide. So far, few studies have focused on rotifer diversity patterns during the impoundment of newly constructed reservoirs. The aim of the present study

was to identify the rotifer taxa during the impoundment of a reservoir (Moglice Reservoir in Albania). Rotifer samplings, near the deepest point of the reservoir, were carried out monthly for a whole year (November 2019 to October 2020) and biweekly during the warm period (May-October). Overall, bdelloids and 51 monogonont taxa were identified. The Brachionidae family was the most diverse with 10 recorded taxa, followed by Lecanidae and Lepadellidae with 5 recorded taxa. The majority of the species (67%) had frequency of occurrence below 20% and were characterized as rare. The co-existence of two different communities, the riverine and the lacustrine, associated with the transition from a lotic to a lentic environment as well as the rotifer's high dispersal ability enabling them to colonize newly established environments explain the high obtained diversity and the high number of rare species. Further study will reveal which of the species will establish stable populations. Rotifera abundance ranged from 0.035 (April 2020) to 12.98 ind/L (December 2019) with various genera dominating. The rest of the biotic communities (cladocerans, copepods and phytoplankton) along with abiotic parameters will be used to identify the factors influencing the rotifer community.

Effects of selenium supplementation on the favorable metabolism pattern alterations and antioxidation response in the rotifer *Brachionus plicatilis*

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Rotifers are frequently used as a model organism for ageing research. The results showed that the lower Se concentrations positively promoted green algae *Chlorella* sp. growth and acted as antioxidant by inhibiting lipid peroxidation (LPO) and intracellular reactive oxygen species (ROS). We found that supplementation of both acid soluble Se at low concentrations and the enriched Se *Chlorella* (Se-*Chlorella*) enhanced rotifer lifespan, increased the rate of spawning, and maintained the stability of mitochondrial membranes. Selenite, which is more toxic than Se-*Chlorella*, is less readily accumulated and biologically active. The results showed that the differentially expressed proteins were primarily involved in anti- ROS and LPO, selenocompound metabolism, glycolysis, and amino acid metabolisms. The ROS level of rotifers was diminished after Se-*Chlorella* feeding, indicating that Se-*Chlorella* could help rotifer *Brachionus plicatilis* to enhance amino acid metabolism and shift energy generating metabolism from tricarboxylic acid cycle to glycolysis, which leads to reduce ROS production. These studies demonstrate the anti-aging effects of Se-*Chlorella* on *B. plicatilis* and to provide a possible approach for enhancing rotifer lifespan.

Impact of different restoration methods on the zooplankton assemblage in the Adriatic coastal ponds

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Zooplankton is an important component of the biocenoses in many lentic ecosystems and is also an exceptional indicator of changes in environmental variables and ecological status of the ecosystem. Anthropogenic impact and global climate changes reduce the number of shallow water bodies, and in order to preserve them, several restoration methods are being carried out. The main objectives of this study were: (i) to compare zooplankton assemblages between unrestored Adriatic coastal ponds of anthropogenic origin (AN) and those subjected to various restoration methods: macrophyte restoration (MR); drainage of sediment (DS); and (ii) to determine the main drivers of the zooplankton composition in each category of ponds. In nine ponds, 42 taxa were determined, and rotifers (26 taxa) mostly contributed to the zooplankton diversity. The greatest abundance of zooplankton was observed in shallow lakes which were restored by macrophyte reduction, while the smallest abundance of zooplankton was observed in shallow water bodies without applied restoration methods. Conductivity, concentration of suspended organic matter, complexity of macrophyte structure and phytoplankton biomass had a significant influence on the zooplankton assemblage. The results of this study confirm the importance of proper implementation of restoration measures in order to preserve biodiversity and maintain good ecological status of shallow water bodies.

Rotifera functional groups in Lake Dojran (North Macedonia) – assessing tool of ecosystem functioning

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The functional feeding groups of rotifers as biological indicators in different water bodies are increasingly studied. The aim of this study was to assess the spatial and temporal patterns of Rotifera functional feeding guilds in relation to the environmental conditions in Lake Dojran. Pelagial rotifers of high abundance (110 ± 98 ind./L) obtained remarkable share (78%) in the zooplankton assemblage. They consisted of 18 species, where ten species are microphagous and was mainly comprised of the most abundant rotifers *Brachionus diversicornis* and *Keratella quadrata* in summer and autumn. The other 8 species are raptorial and have a significantly lower abundance. The microphagous rotifers occupied water layers from the surface to 4 m depth while raptorials prefer surface layers due to low transparency of only 0.5 to 1.8 meters. A correlation analyses revealed significant relationship between the microphagous rotifer abundance and the concentration of nutrients (TP and TN). The dominance of microphagous rotifers and other zooplankters indicated increased productivity in the shallow Lake Dojran. The findings of this study reveal that the functional group approach can be useful

assessing tool of ecological processes in the shallow freshwater ecosystems.

Indirect effects of invasive and native predatory copepods on the population growth of brachionid rotifers

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In Mexico exotic cyclopoid copepod species are common: *Thermocyclops crassus*, *Mesocyclops aspericornis*, *Mesocyclops thermocyclopoides* and *Mesocyclops pehpeiensis*. In this work we quantified the effect of two cyclopoids, an invasive (*Mesocyclops pehpeiensis*) and a native (*Mesocyclops longisetus curvatus*) species on the population growth of *Brachionus calyciflorus* and *Brachionus havanaensis*. We hypothesized that the invasive species would exert a stronger indirect effect than the native species, leading to decreased population growth and egg numbers of the rotifers. Population growth studies of *B. calyciflorus* and *B. havanaensis* were conducted separately, and in the indirect presence of the copepods. The predators were separated from test rotifers by mesh (50 μm) cages placed in experimental jars containing 80 mL synthetic medium. Twenty females of *B. calyciflorus* or *B. havanaensis* were added to the medium containing 500.000 cells/mL of *Chlorella vulgaris*, with two females of *M. pehpeiensis* or *M. longisetus curvatus* present in mesh cage. In all, there were four treatments (*B. calyciflorus* controls, with empty mesh cages in the test vessels, *B. calyciflorus* with *B. havanaensis* in the mesh cages, *B. calyciflorus* with *M. pehpeiensis* and *B. havanaensis* in the mesh cages and *B. calyciflorus* with *M. longisetus* and *B. havanaensis* in the mesh cages. Four replicates were set up for each treatment. For the population growth experiments with *B. havanaensis*, the experimental design was similar except that we used *B. calyciflorus* as prey. The number of individuals of each rotifer species and the egg ratios were quantified and the medium changed daily until a decline in the population density was observed. The results were discussed with emphasis on the indirect impact of invasive or a native predatory copepods on prey rotifer populations in nature.

Comparative genomics of bdelloid rotifers: insights from analyses of highly contiguous genome assemblies

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Bdelloid rotifers are an ancient and asexual metazoan clade that comprises more than 400 morphospecies. Many species of bdelloid rotifers are able to withstand complete desiccation and are radioresistant. Our recent chromosome-scale genome assembly of the extremotolerant bdelloid rotifer *Adineta vaga* revealed the presence of homologous chromosome pairs as well as signatures of paleo-tetraploidy and extensive horizontal gene transfers. However, the origin of paleo-tetraploidy within rotifers and the extent to which bdelloid rotifer species with low degree of resistance to desiccation and radiation acquire foreign genes remain unclear. Moreover, studies on *A. vaga* have demonstrated how horizontally acquired bacterial genes have become part of bdelloid regulatory systems. This large-scale acquisition of foreign genes may represent a critical factor of bdelloid rotifer adaptation without sexual reproduction, but whether similar genes were acquired in other rotifer species remains obscure. Additionally, the study of the evolution of chromosome structures in bdelloids has been hampered by the paucity of highly contiguous genome assemblies. To fill this gap, we obtained chromosome-scale genome assemblies from various species of bdelloid rotifers and from sexual (or facultative sexual) rotifer lineages, allowing us to perform the first comparative genomic analysis within Rotifera. These newly generated genomic data provide the basis for future evolutionary studies of bdelloid rotifers and pave the way for studying the evolution of chromosome structure in asexual animal species.

Assemblage of bdelloid rotifers in the microbial mats from East Antarctica: The ecological interactions between microscopic phototrophs and invertebrates

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Microbial mats harbor numerous microscopic invertebrates comparable to those found in terrestrial mosses and lichens in Antarctica. Recently, comprehensive studies of the diversity of microbial mats have been conducted using genetic analysis of 16S rRNA and 18S rRNA genes. However, the quantitative biological contribution of each microorganism constituting the microbial mats remains unknown. We surveyed microscopic phototrophs and invertebrates in mats sampled from five lakes and one ephemeral wetland on the Sôya Coast, East Antarctica. We identified seven taxonomic groups of phototrophs, of which five (Chroococcales, Nostocales, Oscillatoriales, Chlorophyceae, Bacillariophyceae) were found at all sites, and three taxonomic groups of

invertebrates (Bdelloidea, Nematoda, Tardigrada). The biomass of all groups was higher in the ephemeral wetland than in the lakes. This indicated that the ephemeral wetland might be the most productive and prolific site for microscopic organisms in the surveyed environments during summer. Among the five lakes, Skallen Ôike had the highest biomass. These findings suggested a higher biomass in eutrophic environments (a marine relict lake, Skallen Ôike and an ephemeral wetland) where nutritional carryover or nutritional flux was expected. In addition, statistical differences in the community structure among the lakes and the ephemeral wetland could be detected based on the biomass compositions of the taxonomic group. The community structure of each lake and the wetland was statistically dissimilar, indicating that the microscopic phototrophs and invertebrates had a unique community structure at each study site.

Reorganization of the serotonin-like and FMRF-amide-like nervous systems during metamorphosis of three gnesiotrochan rotifers

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Reorganization of the nervous system is a frequent occurrence in invertebrates that undergo indirect development. Unlike most rotifers that develop directly, many sessile gnesiotrochans possess a larval stage that undergoes metamorphosis, which involves changes to the corona: it may expand and grow extensive lobes or may be replaced entirely by the infundibulum, which is a completely new head. Here, we examine serotonin-like immunoreactivity (SLIR) and FMRF-amide-like immunoreactivity (FMRF-IR) in *Collotheca ferox, Lacinularia flosculosa,* and *Acyclus inquietus.* We hypothesized that the organization and/or expression of specific neural phenotypes in these three species may be reduced after metamorphosis based on prior observations of other rotifers. Immunohistochemistry and confocal laser scanning microscopy were used to map the distribution of both neurotransmitter expression patterns. SLIR results revealed that expression patterns in the cerebral ganglion were variable between species: arch- shaped in *C. ferox* and x-shaped in *L. flosculosa*. The significance of these differences remains to be determined. One commonality was the reduction in number of SLIR neurons after metamorphosis; expression was reduced from six neurons to four in *C. ferox* and from six to two in *L. flosculosa*. This is also likely to occur in *A. inquietus*, but results are pending as are FMRF-amide experiments. So far, findings are similar to previous studies that have shown a reduction in the expression of SLIR neurotransmission after metamorphosis, implying that a functional change has likely occurred due to the adoption of a new lifestyle (from a non-feeding planktonic larva to a benthic and sessile feeding adult).

Community characteristics of rotifers in the different water bodies in Pearl River Delta (China): morphological identification *vs.* environmental DNA metabarcoding

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Rotifers are essential invertebrates in water ecosystems. The community structure of rotifer reflects the environmental changes of the water bodies. To study the community characteristics of rotifers in the Pearl River Delta, we investigated the community structure of rotifer in the different water bodies including lakes with or without cultivated aquatic plants, the Guangzhou section of the Pearl River, and brackish water areas (the Pearl River estuary and Mangrove Nature Reserve in Qiao island, Zhuhai), based on morphological identification and environmental DNA metabarcoding. A total of 101 species of rotifers belonging to 30 genera were recorded. The lakes with aquatic plants had the highest number of rotifer species (67), followed by the Guangzhou section of the Pearl River (60), lakes without aquatic plants (51), and brackish water areas (48). The dominant species were *Polyarthra vulgaris, Brachionus angularis*, and *Trichocerca* spp. Types of water bodies, salinity and chlorophyll-a were the main factors affecting the community characteristics of rotifers. Water connectivity and aquatic plant cultivation can increase rotifer biodiversity. There were 82 species of rotifers recorded by morphological identification and 31 OTUs of rotifers based on environmental DNA metabarcoding technology. Only 40% (2 orders), 42.9% (9 families), 29.7% (11 genera) and 4% (4 species) were shared by the two identification methods, respectively. Primers and imperfect rotifer databases may be responsible for the low annotation of OTU species. Environmental DNA metabarcoding technique is promising for future large-scale investigation and diversity of rotifers.

Species diversity of bdelloid rotifers in leaf litter reveals twenty-five new species records for China

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Bdelloid rotifer survey in leaf litter habitats was conducted during 2017-2021 from 10 provinces of China. In total, 49 morphospecies were identified in this study, representing about 8% of the currently known global bdelloid diversity. These belonged to 10 genera and 3 families, including first-time evidence of 25 species and 3 genera (*Bradyscela, Otostephanos* and *Scepanotrocha*) for China. The phylogenetic position of *B. clauda* was verified in COI sequence and morphological data. The results suggest that broader sampling followed by integrative analysis of morphological and molecular data would identify considerably higher levels of bdelloid diversity in entire China. This should increase our understanding of habitat preferences by bdelloid rotifers. The example of bdelloids further illustrates that greater efforts are required to survey the microscopic species in China as a whole.

Essays under laboratory lights underestimate the toxicity of nanomaterials: A case study of nano titanium dioxide to *Brachionus calyciflorus* under simulated sunlight

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Nano-sized titanium dioxide ($nTiO_2$) generally shows low toxicity to organisms under light- emitting diode (LED) lights. However, its toxicity may increase under sunlight due to photocatalysis-induced oxidative stress. To test this hypothesis, we used xenon lamps to simulate sunlight and compared toxicity of $nTiO_2$ to *Brachionus calyciflorus* under simulated sunlight and LED light. The LC50 of $nTiO_2$ under LED and simulated sunlight were 23.44 and 11.18 mg/L, respectively. Compared with LED light, 10 mg/L $nTiO_2$ significantly decreased lifespan, reproduction, population growth rate, and swimming speed, increased SOD, CAT activities, MDA content and ROS level in rotifers under simulated sunlight. These results indicate a significant enhancement of simulated sunlight on $nTiO_2$ toxicity. In addition, in the $nTiO_2$ treatment under simulated sunlight we observed enhanced ROS production especially around the corona and digestive tract, possibly reducing swimming speed and energy uptake. Exposure to vitamin C effectively alleviated the harmful effects of $nTiO_2$ on rotifer swimming speed under simulated sunlight. These results suggest that increased oxidative stress might be the major mechanism underlying simulated sunlight-enhanced $nTiO_2$ toxicity. Overall, the present study demonstrates that previous laboratory assays under LED lights may underestimate the toxicity of nano-sized materials.

Prospective neotype candidates for a future redescription of the troublesome bdelloid rotifer species *Adineta vaga* (Davis, 1873)

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Örstan (2020) expertly summarises several longstanding taxonomic problems with the species *Adineta vaga* (Davis, 1873). For decades, this name has commonly been assigned to animals collected worldwide, giving the impression of an easily identified, cosmopolitan species. However, there are no clear morphological or molecular criteria to delineate *A. vaga* as a consistent taxonomic entity, or to distinguish it from several equivocal subspecies. Previous analysis of molecular barcodes indicates that the name is associated with dozens of independently evolving entities of polyphyletic origin. This ambiguity is problematic given the growing body of genomic and genetic literature that refers to *A. vaga* variously as a model species, a species complex, a morphological variety or a disparate set of entities posited to share genetic material via speculative interspecific mechanisms. A suggested solution for at least one taxonomic element of this problem would be to redescribe *A. vaga* according to the rules of the International Code of Zoological Nomenclature, by fixing the name on a modern neotype from the original type locality: the grounds of a Victorian house in rural England. Here, I describe recent contacts with the current owners of this house, and provide updates on efforts to sample, culture and

characterise possible candidate lineages. My goal is to consult the international rotifer community about how best to proceed with any potential redescription, in light of molecular and morphological features of the sampled clones and their relationship to lineages designated as *Adineta vaga* in key publications and long-term laboratory cultures.

Combined effects of warming and imidacloprid on survival, reproduction and population growth of *Brachionus calyciflorus*

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The neonicotinoid insecticide imidacloprid (IMI) is used in China for a variety of crop protection purposes, and may contaminate aquatic ecosystems via spray drift, surface runoff and ground water leaching. The chronic toxicity of IMI to aquatic animals may be affected by warming. To determine the combined effects of warming and IMI on the survival, reproduction and population growth of *Brachionus calyciflorus* s.s. Pallas, a cohort life table experiment was conducted at three temperatures (20, 25 and 30°C) and eight IMI concentrations (0 (control), 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 mg/L). The results showed that compared with controls, treatments with higher IMI concentrations significantly inhibited the survival (measured as life expectancy at hatching), reproduction (net reproductive rate) and population growth (intrinsic rate of increase) of rotifers at each temperature. The inhibiting effect on survival increased with increasing temperatures, but those on reproduction and population growth increased only when the temperature was up to 30°C. Treatments with lower IMI concentrations stimulated survival, reproduction and population growth of the rotifers at each temperature, except 25°C for which lower IMI concentrations did not affect intrinsic rate of increase. The stimulatory effects on survival, reproduction and population growth were the most obvious at 20°C, but the least at 25°C. The proportion of mictic offspring was affected only by temperature. Our results indicate that when monitoring the ecological effects of sublethal concentrations of IMI, environmental temperature and possible adaptation of the rotifers to it should be taken into consideration.

The resistance capability to dehydration in bdelloid rotifer *Rotaria rotatoria*

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Anhydrobiosis is one of the important capacities of bdelloid rotifers to tolerate unfavorable environmental stress. However, in order to determine their survival and recovery there is scarce information about the role of endogenous (strains) and exogenous factors (humidity, duration of their dehydration and

rehydration). In this study, the resistance capability of different strains of bdelloid rotifer *Rotaria rotatoria*, related to relative humidity and duration of dehydration and rehydration is investigated. Our results revealed that the survivorship of dehydrated rotifers decreased with increase of dehydration duration and reduction of relative humidity. The strain, relative humidity and dehydration duration all had significant effects on the recovery rate of *R. rotatoria* through 1h rehydration, while the effects of strains and relative humidity on the recovery rate slightly declined for rehydration period of 12h and 24h. The recovery rate was lowest after long dehydration and short rehydration duration, while increased by shorten the dehydration duration and extending the rehydration procedure. Among three strains, ZJ27 showed higher survival rates than the other two, which could be attributed to the frequent drought and rainstorm of tropic habitat in Zhanjiang City nearby the sea. Accordingly, species, originating from different habitats, experienced different desiccation frequencies in their adaptive evolutionary history, resulting in certain species with different anhydrobiotic capabilities, and relative humidity acts as a key factor in resisting desiccation.

Diversity and distribution of rotifers in the heterogeneous habitats in Guangdong Province (China)

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Rotifers are widely distributed in various types of waters or terrestrial habitats. Guangdong Province in the southern China, with a tropical and subtropical climate, is known through the spatially and temporally heterogeneous habitats. According to our survey and the collected data, a high diversity of rotifers (64 genera, 349 morphospecies and two new species, *Lecane zhanjiangensis* sp. nov. and *Colurella ovalis* sp. nov.) were recorded from Guangdong province during 2000-2022. However, they are unevenly distributed in different aquatic and terrestrial ecosystems. The monogonont rotifer species diversity (255) was highest in the urban habitats presented by city river segments (184), followed by lakes (134), reservoirs (130), estuary and coastal waters (65), aquatic plants (42), and the lowest was in ponds (20). Urban segments of the Pearl and Han rivers were almost lentic and eutrophic, thus providing abundant nutrients and various microhabitats for rotifer populations. Results showed a significant correlation between α diversity and flow rate, and revealed the important effect of habitat heterogeneity. Moreover, 94 species of bdelloid rotifers were found (mosses 56, leaf litter 44, lichen 13, streams 10, aquatic plants 7, lakes and rivers 6, ponds 3), indicated a high diversity of bdelloid rotifers in terrestrial habitats such as mosses, leaf litter and soil. Conclusively, habitat heterogeneity is the most important factor that regulates rotifer biodiversity

Between big and small – how conditions of temperature-size rule performance dictate optimal body size: The case of *Lecane inermis*

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The temperature-Size Rule (TSR) is a well-established phenomenon to describe the growth response of ectotherms to temperature. TSR predicts a negative correlation of body size to increasing temperature. Nevertheless, there are limits to plastic body size response. In consequence, the TSR could only be applied within a specific optimal thermal range which is determined by the minimum and optimum temperatures for performance directly referred to populations of *Lecane inermis* (Rotifera, Monogononta). Each population had known thermal preference, being either generalist or specialist; cold- or warm-preferring. Each population of *L. inermis* was exposed to six thermal regimes from 10°C to 35°C. The response trait was body size. Additionally, population growth rate was estimated as a fitness measure to establish the population-specific optimal thermal range and to enable the interpretation of the significance of possible differences in TSR patterns-. The results confirmed predictions that the populations prefer cooler conditions exhibited ranges of TSR shifted to lower temperatures, whereas the populations prefer warmer conditions achieved ranges of TSR shifted to higher temperatures. Optimal thermal ranges for the TSR differ with thermal preferences of examined populations. Results show that it is important to take thermal preferences into account while planning the studies on the plastic body size response to temperature changes. Even closely related organisms may differ in the thermal ranges within which they are able to plastically respond to environment.

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