# Cladocera XII

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# KEYNOTE LECTURES

## Unveiling the ecological impact of environmental threats through model organisms

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Microplastic and nanoplastic pollution is on increasing rise, posing threats to ecosystems and human health. While research on these pollutants often focuses on their direct toxicological effects on individual organisms, their broader ecological ramifications, particularly on species interactions, remain largely unexplored. In my talk, I will delve into how *Daphnia* and cyanobacteria serve as ideal model organisms for unraveling the multifaceted impacts of micro- and nanoplastics, as well as other environmental stressors, on species interactions, including pivotal relationships such as host-parasite dynamics. By harnessing the ecological significance of both systems, we can illuminate the hidden consequences of plastic pollution on freshwater plankton communities. I will present our latest findings and discuss the crucial research gaps that need to be addressed to fully comprehend the ecological implications of emerging pollutants.

# To be or not to be a model organism – *Daphnia* in evolutionary ecology

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A closer integration of the fields of ecology and evolution is viewed by many as inevitable to understand, evaluate, and predict the effects of human activities on ecosystems. Experimental research plays a key role in this endeavour. However, much of this experimental research in freshwater ecosystems relies on a well-established model organism: our beloved *Daphnia*. Undoubtedly, *Daphnia* provide unique opportunities to study the interactions between ecological and evolutionary dynamics. Join me here in exploring what we have learned from eco-evo experiments with *Daphnia* and what may be not.

# ORAL PRESENTATIONS

## Chemical signaling in aquatic ecosystems: Quantifying the fish kairomone dynamics in order to assess its reliability as indicator for predation risk in *Daphnia*

🕮

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In aquatic ecosystems, the use of chemical cues by prey for predator detection and subsequent predator avoidance behaviors is a well-explored phenomenon. One well-studied example for is the Diel vertical migration (DVM) in the freshwater microcrustacean *Daphnia* is triggered by chemical cues, also referred to as kairomones, by planktivorous fish. Only recently a first DVM-inducing kairomone from fish has been identified as 5-alpha-cyprinolsulfate (CPS). Due to the high costs associated with DVM, the kairomone should reliably indicate the risk of predation. To do so, CPS concentrations should closely reflect fish abundance or fish activity, which leads to the hypothesis that CPS may show a high turnover rate. Here we have measured in-situ concentrations of CPS over the course of a season by using HPLC coupled to high-resolution mass spectrometry in order to assess if CPS concentrations vary with the abundance of planktivorous fish. However, seasonal variation of CPS may be low and not necessarily reflect the rate of CPS turnover. In order to assess rates of CPS turnover we determined rates of CPS exudation by fish and rates of bacterial CPS degradation. Using the latest techniques of metabolomics, we delve into the search for degradation products of CPS. Uncovering bacterial degradation products and turnover rates of the kairomone is crucial, as it provides insights into the fate and persistence of CPS in the ecosystem, ultimately enhancing our ability to predict predator risks associated with DVM in lakes.

**Keywords:** diel vertical migration (DVM); kairomones; predator-prey; bacterial degradation

# A new water flea *Daphnia mariae* (Branchiopoda: Diplostraca) from South America

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*Daphnia mariae*, a new species, has been found in a small temporary lake in northern Chile. This species is morphologically and genetically close to *D. peruviana* Harding, 1955, discovered in mountain ponds in southern Peru. Both species are large sized, belong to the subgenus *Daphnia* and, so far, are exclusive to South America. Morphological descriptions and differential diagnosis are given for the two species. Diagnostic traits differentiating *D. mariae* sp. nov. from *D. peruviana* can be seen in the caudal spine of the male and female adults and juveniles, the dorsal curvature of the adult female valves, the number and thickness of teeth in the anal portion of the postabdomen and the length of the male seta of antenna I. A phylogenetic analysis with a fragment of the Cytochrome Oxidase I (COI) is also provided which confirms the morphological results. *D. mariae* and *D. peruviana* are separated by 14.5 % differentiation. The genetic analyses also showed that previous analyses of *Daphnia* from Argentina also belong to *D. mariae* indicating that the new species is distributed at least in these two countries.

**Keywords:** *Daphnia*; taxonomy; Chile; Peru; genetics

# Genetic and environmental determinants of longevity in a model zooplankton organism: a meta-analysis

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Longevity and aging studies require knowledge about intraspecific variation for lifespan for two reasons. First, we need reliable control data in experiments that aim to measure effects of various lifespan interventions. Second, genetic and physiological determinants of longevity may allow us to elucidate mechanisms of extended lifespan. We studied longevity of 12 genotypes of *Daphnia magna* from different geographic locations and habitat types and measuring two parameters of lipid peroxidation (LPO) – amounts of primary products of LPO in 4 different tissues and of MDA, the final product of LPO in whole body extracts. All three measured parameters changed with age; LPO decreasing with age, while MDA concentration increasing with age. The 12 genotypes studied significantly differed in all three parameters. LPO and MDA measurements correlated with longevity only in long-lived genotypes, but not in short-lived ones. Median lifespan was positively correlated with the LPO levels measured in all four tissues, in any age classes. This indicates that clones capable of accumulation or synthesis of polyunsaturated fatty acids, the main target of LPO tend to live longer. Lifespan was positively correlated with MDA levels observed early in life, but negatively correlated with those observed in mid-life age class (MDA * Age interaction, $P < 0.004$). We hypothesize that this may reflect early life benefits of accumulating PUFAs, followed by detrimental effects of accumulation of toxic and mutagenic LPO products in tissues.

**Keywords:** *Daphnia magna;* Lipid Peroxidation; MDA

# Does the fitness of *Daphnia* of different body sizes vary differently in response to changes in temperature and food quality?

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Both poor food quality (e.g. presence of cyanobacteria) and rising temperature tend to favour cladocerans of small body size. Thus, those two factors should affect the fitness of *Daphnia* in a size-dependent manner. To test this assumption, we determined the juvenile growth rate (gi; a reliable measure of cladoceran fitness) of large-sized *Daphnia magna*, medium-sized *D. pulex* and small-bodied *D. longispina*, each represented by 3 clones (genotypes), exposed to different diets and temperature regimes. Daphnids were reared in a flow-through system with constant food concentration (~0.5 Corg L-1) at low (18ºC) and high temperature (24ºC). There were three food regimes (1) a suspension of the green alga *Acutodesmus obliquus* with a stoichiometric carbon to phosphorus ratio optimal for *Daphnia* (C:P ≈ 120), (2) a suspension of *A. obliquus* with a stoichiometric ratio of C:P ≈ 800 indicating severe phosphorus deficiency, and (3) a mixture of *A. obliquus* (10%) and the cyanobacterium *Synechococcus elongatus* (90%) which is deficient in sterols and unsaturated fatty acids. Temperature and diet quality affected the fitness of *Daphnia* in an interactive way, however no clear effect of body size on susceptibility to low quality diet was detected. We conclude that factors other than food quality are responsible for the absence of large-bodied daphnids in warm subtropical and tropical lakes.

**Keywords:** global warming; stoichiometric C:P ratio; cyanobacteria; green algae; somatic growth rate

This study was supported by a grant from the National Science Centre, Poland, project no 2019/33/B/NZ8/01567.

# The combined effects of coinfection and temperature on the planktonic crustacean *Daphnia magna*

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Changes in water temperature can strongly impact the biotic environment of aquatic ecosystems. Zooplankton are particularly susceptible to these changes, due to their inability to regulate their body temperature. Consequently, their endoparasites are also affected by temperature variations, influencing the parasites' ability to exploit their hosts, which further depends on host susceptibility and parasite resilience. In natural populations of the crustacean *Daphnia magna*, it is common for individuals to be infected by multiple parasite species. Our study aimed to understand how interactions between parasite species are influenced by the surrounding temperature. To this end, we exposed newborn D. magna individuals to four temperature conditions: 16°C, 20°C, 24°C and 28°C, adjusting exposure age to physiological age using Degree Days (DD). At each temperature, groups of individuals were exposed to either a single parasite or two parasite species, sequentially (at 120 and 180 DD) or simultaneously (at 120 DD), using the fungi *Metschnikowia bicuspidata* (*Mb*) and the microsporidium *Hamiltosporidium tvaerminnensis* (*Ht*). Our findings showed no coexistence by the two parasites. However, the type of exposure (single, sequential or simultaneous) and temperature significantly affected infection traits. Simultaneous exposure to *Ht* reduced the proportion of *Mb* infections at 16°C and 20°C compared to single and sequential exposures. Additionally, simultaneous exposure at 28°C resulted in the fastest mortality (i.e., time-to-host-death) among all treatments. These results demonstrate that complex biotic systems respond differently to abiotic factors than simpler systems, underscoring the need for further research on the interactions between complex biotic systems and their abiotic environments.

**Keywords:** climate change, coinfection, *Daphnia magna*, temperature, parasite

# Diversity of Cladocera-associated microbial communities across four alpine Italian lakes

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Microbes play a crucial role in the well-being of many hosts, not only humans but also plants and animals. The composition of the microbiota associated with different species of Cladocera is of particular interest to highlight the host-microbe interaction and its ecological implications in lakes. Moreover, Cladocera-associated microbiota may exert significant effects on biogeochemical cycles, especially in remote and freshwater ecosystems that are sensitive to changing climate. To widen our knowledge of Cladocera-microbiota interaction, we investigated populations of dominant Cladocera species as hosts from four high mountain lakes: two lakes in the Central Alps (Paione Superiore and Inferiore) and two lakes in the Northern Apennines (Santo and Scuro). These lakes are part of the Italian LTER (Long-Term Ecological Research) network. Despite several published studies on zooplankton dynamics, nothing is known about the microbes associated with Cladocera in these high altitude lakes. We sampled various species of Cladocera (*Daphnia longispina, Diaphanosoma* sp., *Pleuroxus* sp.) and copepods. The aim of this study was to investigate potential intraspecies and interspecies or habitat-based patterns. We employed two types of analysis: firstly, we performed DNA barcoding analyses for each morphological host species; secondly, we conducted amplicon sequencing of the 16S rRNA gene on pooled samples (consisting of 20 – 30 individuals) of the same species and on pooled samples of the whole zooplankton community from each lake. Our results are the first to reveal species-specificity of microbiomes across different species of freshwater Cladocera high altitude lakes.

**Keywords:** biodiversity; DNA; microcrustaceans host; mountain lakes

# Caloric restriction extends lifespan in long-lived, but not short-lived genotypes in *Daphnia*

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Caloric restriction (CR) is known to extend life in a variety of organisms including yeasts, worms, flies, mice, and, by some account, primates. *Daphnia*, a classic model organism for developmental biology and ecophysiology, was one of the first organisms in which the life-extending effect of CR has been demonstrated. However, more recently the magnitude or even existence of the CR effects in *Daphnia* has been controversial, partly because the effect is known to be stronger in some genotypes than others, and because excessive caloric restriction (e.g., starvation) can have the opposite effect on longevity. We conducted a series of lifespan experiments in 12 different genotypes of *D. magna* that included both short-lived and long-lived ones, and covering a broad range of food levels, including the level above the ad libitum level. We show that the highest lifespan is observed between food levels of 5x104 and 3x105 algae cells per mL, over which range the lifespan is not changing much, although in most genotypes the longest lifespan is observed at 105 cells/mL. The 4-fold increase in food concentration to 4x105 cells/mL results in a significant (~30%) reduction of lifespan in the long-lived genotypes, but causes no difference in lifespan of short-lived ones. This lifespan difference showed little or no negative correlations with fecundity in each food level, inconsistent with the hypothesis of lifespan reducing effect of elevated reproduction under high food. We hypothesize that lifespan-reducing genomic determinants present in the short-lived clones eliminate the beneficial effects of caloric restriction.

# Microturbulence-induced helmet formation and interclonal variability of phenotypic plasticity in two species of *Daphnia*

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Helmets are widespread defensive structures in *Daphnia*, formed in response to kairomones produced by various predatory taxa, or to abiotic stimuli including microturbulences. While the response to kairomones is clearly an anti-predator adaptation, the importance of the response to physical stimuli is less clear. In *D. cucullata*, we previously observed striking differences in phenotypic responses between clones from two localities differing in the presence of the pelagic predatory cladoceran *Leptodora* (which produces particularly strong vortices while swimming). Clones from a *Leptodora* site formed large helmets in response to microturbulences, whereas those with no experience of this predator showed very weak response, although all clones formed helmets when kairomones of *Chaoborus* were present. To test generality of this pattern, we exposed multiple clones of *D. cucullata* and another unrelated helmet-forming species, *D. (Ctenodaphnia) sinesis,* originating from ecologically different habitats to multiple stimuli including microturbulences and kairomones of relevant predators. In *D. sinensis,* morphological changes were induced only in response to notostracan (*Triops*) kairomone. Significant interclonal variability in kairomone-induced helmet growth was observed whereas induced changes in spine length was much less variable. In *D. cucullata*, helmet size and spine length changed in response to *Chaoborus* kairomone but not the other stimuli. In both species reactions to some of the other stimuli (including microturbulence in *D. cucullata*) have been documented previously, its complete absence in our experiments was thus unusual. It might be explained both by interspecific differences and by insufficient intensity or character of the stimuli.

**Keywords:** antipredator phenotypic plasticity; helmet; microturbulence; local adaptation

# Food thresholds in *Daphnia* revisited: do they depend on body size?

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Size-efficiency hypothesis (SHE) implies that large-bodied cladocerans species should have a competitive advantage over small-bodied ones due to higher efficiency in utilizing resources. Large-bodied species are expected to outcompete small-bodied ones when food quality is good due to lower population threshold food concentration (TFC), and vice versa when food quality (in terms of stoichiometry or essential lipids) is poor. Small-bodied species are also believed to be superior in elevated temperatures. However, simultaneous effects of food quality and temperature on TFC of differently sized cladocerans have not been tested. In laboratory experiments, we tested the predictions of SHE by determining TFCs of three species of *Daphnia*, each represented by three clones, that differed with regard to body size. Daphnids were raised in two temperatures (18°C and 24°C), in gradients of food quantity that differed with regard to essential lipid content or stoichiometric ratios. We found that TFCs of the tested species were not related to body size. The limitation by essential lipids, but not by C:P ratios, affected TFCs of the tested species. Temperature modifies the effects of food quality on TFCs. At elevated temperatures, the superiority of large-bodied species diminished when food quality was good, whereas under poor biochemical quality at elevated temperatures the largest species performed better than the smaller ones.

**Keywords**: interspecific competition; body size; biochemical food quality; stoichiometric ratios; temperature

The study was supported by NCN: NAWA BPN/SZN/2021/1/00023/U/00001and OPUS 2019/33/B/NZ8/01567

# Influence of encounter rate, barrier resistance, and internal clearance on the successful infection of *Daphnia dentifera* by *Metschnikowia bicuspidata*

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The variation in the succeptability of *Daphnia dentifera* to infection by the fungal pathogen *Metschnikowia bicuspidata* is driven by both the host genotype and the environment. To complete its life cycle, *Metschnikowia* must encounter a foraging host, and overcome the host's barrier resistance (the gut wall) and the host's internally mounted immune response (internal clearance). We have conducted several laboratory experiments to determine the stage of this host-pathogen interaction at which various environmental factors (e.g., pesticides, temperature, resources) may interact with host genotype to determine susceptibility. We investigated the encounter rate by quantifying gut passage time and the number of spores that enter the gut. We then quantified the proportion of spores that successfully entered the body cavity (barrier resistance), and the hemocyte response to spores entering the body cavity (one metric of internal clearance). Although we have support for each factor influencing susceptibility, the primary driver often depends on the particular host genotype by environment interaction.

**Keywords**: disease; parasite; fungus

# Indicators of climate-driven changes in long-term zooplankton composition in Lake Maggiore (Italy)

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Zooplankton is a key component of lake food webs and a prompt indicator of changes occurring in ecosystem structure and functioning. Changes in zooplankton communities with lake eutrophication and its subsequent reversal have provided a basis for identifying biotic indices characterizing and quantifying the impact along a pressure gradient or over time. The increasing concern of climate change and its impact on lake zooplankton communities brings a new challenge for novel indices in order to detect impacts and mechanisms affecting the functioning of pelagic ecosystems. In the present study, we examined the zooplankton community of the large deep subalpine Lake Maggiore (Italy) during a period of about 40 years, covering changes in both trophic conditions and climate warming. Using monthly data of the upper 50 m depth layer, we investigated the application of both common and novel zooplankton biomass-based indices in order to provide a better understanding of changes in the lake ecosystem over time. Examining annual and seasonal patterns of different zooplankton taxa and groups, we observed over time a decreased contribution of *Daphnia* during the summer and a concurrent increase of microzooplankton, suggesting a change in grazing/phytoplankton control in the lake during the recent period. Our study, confirming that zooplankton communities integrate environmental changes, indicates the importance of seasonality in investigations and the inclusion of the whole size range of zooplankton in the interpretation of lake ecosystem functioning in response to trophic and climatic changes.

# Impact of global warming on parasitic infections of *Daphnia:* Can thermal adaptation of fungal spores extend infectivity time window?

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Global warming increases risk of parasitic infections as elevated temperatures can enhance the performance of parasites against their host. Many parasites have environmental transmission stages that are directly exposed to abiotic conditions, making thermal adaptation crucial. Given that parasite responses are rarely measured independently of their host environment, most available data report composite measures, leaving the specific changes in host–parasite interactions along environmental gradients unclear. Elevated temperatures can exceed the parasite's upper critical limit, potentially reducing its infectivity and survival. We hypothesized that free-living parasite forms transferred to warmer environment would exhibit a reduced infective period at higher temperatures, whereas those maintained at higher temperatures would adapt, resulting in higher infectivity than controls. To test this, we conducted an experimental evolution assay with a fungal parasite of *Daphnia magna*, *Metschnikowia bicuspidata*, that produces free-living spores. Parasite evolutionary lines were maintained at elevated (24°C) and control (20°C) temperatures over 20 parasite generations. We tested the performance of spores from each line after 1, 4, 8, 12 and 16 days of incubation in water under both elevated and control temperature conditions. Preliminary results indicate that virulence of *Metschnikowia* lines kept at 20°C gradually decreases over time, while those kept at 24°C maintain consistent virulence until a sudden drop on day 16. The infectivity and infection intensity of various combinations are still being analyzed, but these findings suggest that thermal adaptation plays a significant role in the parasite's ability to maintain virulence under elevated temperatures.

**Keywords**: global warming; *Daphnia*, fungal parasite;

# Eco-evolutionary hotspots in a heterogeneous landscape – an experiment in a sodic Hungarian pondscape

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There is much literature on evolutionary trait change significantly affecting ecological processes. Most studies, however, focus on simplified settings, and it is therefore less clear how important eco-evolutionary interactions are in nature. We here report on results of an in-situ transplant experiment of populations of the water flea *Daphnia magna* across ponds that differ in salinity, using a dense sodic bomb crater area in the Hungarian pusta as a model system. We show that eco-evolutionary feedback is not important in low salinity ponds, whereas local trait adaptation has strong ecological consequences in high salinity ponds, resulting in slower population development and reduced suppression of phytoplankton. Top-down control of phytoplankton is dictated by ecology (i.e. the presence/absence of *Daphnia*) in low but by evolutionary trait adaptation in high salinity ponds. Similar pond-dependent eco-evolutionary feedback was observed for resistance to immigrant regional zooplankton. Our results suggest the presence of eco-evolutionary hot and cold spots in landscapes. We show that these eco-evolutionary dynamics can dampen ecological differences among habitats.

# Hierarchical eco-evo dynamics mediated by the *Daphnia* gut microbiome

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The concept of eco-evolutionary (eco-evo) dynamics, stating that ecological and evolutionary processes occur at similar time scales and influence each other, has contributed to our understanding of responses of populations, communities, and ecosystems to environmental change. Phenotypes, central to these eco-evo processes, can be strongly impacted by the gut microbiome. The gut microbiome shapes eco-evo dynamics in the host community through its effects on the host phenotype. Complex eco-evo feedback loops between the gut microbiome and the host communities might thus be common. Bottom-up dynamics occur when eco-evo interactions shaping the gut microbiome affect host phenotypes with consequences at population, community, and ecosystem levels. Top-down dynamics occur when eco-evo dynamics shaping the host community structure the gut microbiome. We apply this concept to the *Daphnia* experimental model system.

**Keywords:** hierarchical eco-evo dynamics; microbiome-mediated phenotypic plasticity; host-microbiome interaction; global change

# Predation has driven evolution of *Daphnia*

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Predation is a major driver of the evolution of prey species, however, the genetic bases underpinning the evolution of predator-induced defenses remain largely unexplored. Here, we detected two heritable morphotypes from 64 cladoceran (*Daphnia sinensis*) clones (from 19 populations) across China: morphotype A, characterized by a pointed head-tip (PH) at the first instar stage, a feature exclusively found in large water bodies inhabited by the predator *Leptodora richardi* and that tends to regress in adults if raised with no predator; morphotype B exhibits a flat-topped head (FT) and resides in *L. richardi*-free puddles and ponds. Intriguingly, PH individuals can develop a prominent recurvate helmet in response to predator (*L. richardi*) kairomones. But this is not the case for FT individuals. Subsequent investigation into the genomic variation of these 64 clones revealed pronounced genomic divergence between PH and FT clones/populations. The difference could be partially attributed to genomic signatures associated with the predation regime: a series of candidate SNPs potentially linked to neonate head polymorphism. Notably, gene *TM2D2*, a regulator of the Notch signaling pathway, that controls morphogenetic processes, exhibited strong positive selection in the clones of morphotype A. Our findings indicate a genomic basis for predation-driven population divergence.

**Keywords:** *Daphnia*; genomic evolution; inducible defense; morphotypes; predation

# Mutational foundations for the evolution of phenotypic plasticity

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Phenotypic plasticity is the ability of organisms to express different phenotypes in response to environmental stimuli. Due to the ability to assay genetically identical individuals in different environments and a wide range of responses to environmental stimuli, *Daphnia* have become a major models system in understanding the ecology and evolution of phenotypic plasticity. Most evolutionary research on phenotypic plasticity focuses on existing genetic variation within or among populations, or comparisons among species and the conceptual focus is usually on adaptive processes. Little thought or data has addressed the mechanisms by which phenotypic plasticity originates through spontaneous mutation. Mutation could act to increase phenotypic plasticity if phenotypic plasticity represents a relatively large mutational target, or-if mutations with environment-specific effects destroy fixed phenotypes. Conversely, mutation could act to decrease phenotypic plasticity if phenotypic plasticity is generally adaptive and mutations are generally maladaptive, or because phenotypic plasticity requires complex processes that can be disrupted at many points. We used mutation accumulation assays followed by assays in multiple environments to quantify the effects of spontaneous mutation on phenotypic plasticity of life history and morphology. We found overall, we found no directionality to mutational effects on plasticity. However, particular traits in particular genetic backgrounds did show directional effects. We also found little evidence for mutational correlations between plasticities of different traits, even though these traits are often genetically and environmentally correlated in the wild. Together, our results suggest that evolutionary adaptation of phenotypic plasticity is relatively unconstrained by the availability of spontaneous mutation.

**Keywords:** life history; body size; mutation; temperature; resource availability

# Consuming heat-acclimated algae improves heat tolerance and longevity in *Daphnia*

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Xenohormesis theory suggests that consuming stressed food might provide physiological adaptation to the consumer for similar stresses. To evaluate this, *Daphnia* was provided *Nannochloropsis limnetica* which grows optimally at 10-20℃. We heat-stressed the algae by growing them either at 20℃ or at 25℃, which is, respectively, the upper limit of optimal zone and close to the upper tolerance limit for *N. limnetica* and kept algae grown at 12℃ as the control group. *Daphnia* were also grown at either 12℃, or 20℃, or 28℃. In the acute heat tolerance experiment, *Daphnia* kept at 28℃ and consuming algae grown in 25℃ had significantly higher heat tolerance compared to the *Daphnia* kept in the same temperature but consumed algae grown in 12℃ ($p<0.0001$). Similarly, *Daphnia* kept in 20℃ and fed with heat-stressed (25℃-grown) algae had significantly higher heat tolerance compared to their counterparts fed with algae grown at 12℃, or 20℃, ($p<0.0001$). Additionally, the cohort consuming 25℃-grown food had significantly higher longevity compared to the group consuming 12℃ foods ($p<0.0001$). Finally, in a total antioxidant assay, *Daphnia* consuming 25℃-grown algae had significantly higher levels of antioxidants compared to the *Daphnia* consuming other two diets ($p<0.0001$). This correlates with significantly higher antioxidant levels in heat stressed algae compared to the control ($p<0.001$). A GC-MS study revealed lower levels of Eicosatetraenoic acid in stressed food compared to control which could play a role in reduced membrane fluidity and lower oxidative damage. These novel findings indicate consumption of stressed algae might provide physiological adaptation and longevity.

**Keywords:** *Daphnia*, *environmental stressors,* longevity, heat-tolerance, antioxidants.

# Some things just don't scale up: Qualitatively different impacts of parasites at individual- and population-levels

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Parasites can strongly affect host fitness and we would predict that, all else being equal, more virulent parasites impact host populations more than parasites with only minor effects on host fitness. Our model species, *Daphnia dentifera*, is frequently infected by a variety of parasites. Some, like the fungus *Metschnikowia bicuspidata*, are highly virulent, killing their host within two weeks of exposure. Others, like the microsporidian *Ordospora pajunii,* seem to have a weak effect on individual host fitness. We asked whether those differences in virulence at the individual-level scale up to produce predictable population-level patterns. We hypothesized that host populations exposed to *M. bicuspidata* will have much lower population densities in comparison to unexposed (control) populations, while populations exposed to *O. pajunii* will not differ from the control. Our hypotheses were not supported by the data. Instead, *Daphnia* populations exposed to *M. bicuspidata* did not differ in density compared to the controls despite substantial parasite prevalence. *Daphnia* populations undergoing *O. pajunii* epidemics had much lower densities than controls or *M. bicuspidata* exposed populations. The lack of impact of *M. bicuspidata* epidemics on host population densities might be due to rapid replacement of the individuals killed by the pathogen through reproduction and relaxed competition. The much stronger than expected population-level negative effects of *O. pajunii* are most likely due to transgenerational virulence. We conclude that results of life-table experiments may not scale up, with dramatically different impacts of parasites at the individual and population scales.

**Keywords:** *Daphnia*; epidemics; host-parasite interaction; population dynamics; virulence

# *Daphnia obtusa* as model organism for the interaction between antimicrobial resistant bacteria and zooplankton

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Perialpine surface waters receive a constant influx of bacteria and cell-free DNA containing antimicrobial-resistant genes from wastewater treatment plants, many of which are also present in the microbial communities of lakes throughout the year. While research has primarily focused on the occurrence of such genes, little is known about the ecological interactions of these bacteria with the autochthonous lake community. In recent years, we have studied the interaction between zooplankton and antimicrobial-resistant bacteria, using *Daphnia obtusa* and the microbial community from Lake Maggiore. Due to the highly flexible microbiota composition of *D. obtusa*, it provides an intriguing habitat for resistant bacteria entering the perialpine aquatic system from wastewater treatment plant effluents. These bacteria often struggle to compete in free water due to their lack of adaptation to cold temperatures and low nutrients, but they may find a temporary host in *D. obtusa*, allowing them to adapt and potentially engage in genetic exchange with autochthonous bacteria. Conversely, specific marker genes for antimicrobial resistance are disproportionately reduced in the presence of *D. obtusa*, likely due to their preferential removal of larger bacteria, filaments, and aggregates. Furthermore, we demonstrate that *D. obtusa* and other zooplankton remove cell-free DNA and release enzymes that alter the conformation of this DNA, thereby affecting its uptake by bacteria. Overall, our studies highlight the interplay between antimicrobial-resistant bacteria and *Daphnia* as an intriguing ecological model for understanding the resistance and resilience of aquatic systems to genetic contamination from anthropogenic pollution.

**Keywords**: *Daphnia obtusa*; antimicrobial resistance; Lake Maggiore; microbiota; community assembly

# Cladocera community diversity in an urban multiple reservoir system

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Colentina river is a highly meandered river with oxbow lakes and multiple reservoir system, which flows through Bucharest, the capital of Romania. As Colentina river is part of the Danube's watershed and an urban water body, the river itself and the lakes it forms pose a great interest from an ecosystem services perspective. Cladocera community may be very important in the zooplankton community and the benefits which human society may receive from such ecosystems. Therefore, in the current study we aimed to characterize the Cladocera community structure in relation to the chlorophyll concentrations of green algae, diatoms, cryptophytes and cyanobacteria and of physical chemical parameters. Sampling was done between March and November 2019, at 10 sites on the meandering dammed lakes. The most abundant genera were *Diaphanosoma* and *Bosmina*. *Leptodora kindtii* had low abundances and was present only once in a single site. Concerning the correlation with potential food sources of filter-feeding cladocerans, only green algae chlorophyll showed a positive strong correlation with the Shannon and Simpson diversity of these crustaceans. The abundance of different Cladocera was not correlated with the intraurban or extra urban positioning of the sampling sites. However, future research may provide more information on the spatial and temporal dynamics of Cladocera relative to various pressures which may be developed in urban environments.

**Keywords:** Cladocera community diversity; urban water body

# Between the endemic and the alien: Revision of *Daphnia* from crater lakes El Sol and La Luna, Nevado de Toluca

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The high mountain crater lakes El Sol and La Luna have special characteristics and are located in a hotspot of cladoceran endemism. In these lakes, endemic cladocerans such as *Alpinalona manueli* and *Ilyocryptus nevadensis* were recorded, but the environmental conditions of both water bodies have changed due to anthropogenic activities. Since more than 20 years ago, *Daphnia ambigua* has been recorded for both lakes; however, a deeper revision shows that *Daphnia* sp. (previously determined as *D. ambigua*) from Lake La Luna presents morphological differences from *Daphnia ambigua* s.l., such as high melanization, longer setae in the thoracopods, the first segment of the second antennae's endopod being longer, the spinules on the ventral and dorsal posterior margin absent. Males have considerably shorter antennule, aesthetes, and spine (<0.2 body length). The morphological differences mentioned above make it likely that this is a new species, and molecular information is ongoing. In Lake El Sol, we found an additional species of *Daphnia* not documented in earlier studies. Through examination of both morphological and molecular (COI) characteristics, we observed its close resemblance to *D. pulex*. The introduction of *D. pulex* is not clear, although it was found in samples from 2016 and 2022, but the previously reported *D. ambigua* was not detected. The presence of the alien *D. pulex* represents a threat to local endemic cladocerans, therefore it is relevant to implement conservation strategies.

**Keywords:** COI; endemism; Central Mexico; taxonomy; zooplankton

# Centuries of dominance of a single polyploid *Daphnia* clone

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Many Arctic lakes and ponds harbour populations of *Daphnia* that are polyploid, a trait that is more frequently encountered in higher latitudes or altitudes. Polyploidy in *Daphnia* is generally associated with obligate asexuality, and the resulting clonal lineages are thought to be relatively short-lived with an estimated age of only decades. To test this assumption, we studied *Daphnia* populations from lakes in South-West Greenland. This region has been strongly impacted by climate change in the past decades as well as across the past centuries, profoundly impacting freshwater systems, affecting for example the duration of the growing season, light conditions, and lake productivity. We collected dormant eggs from dated sediment covering the past two centuries and used whole genome sequencing or microsatellite markers. We found lake-specific clonal lineages in three lakes of the area. In contrast to this higher spatial diversity, within-lake genetic diversity was extremely low with a single clone dominating each of two population across the entire time span studied. The uncovered genetic uniformity raises questions about the capacity of these populations to adapt to environmental change. Using a combination of genomic data and results from phenotypic assays of extant and resurrected *Daphnia*, the fate of these populations in the light of projected climate change will be discussed.

**Keywords:** egg bank; whole genome sequencing; microsatellites; resurrection ecology

# Resting eggs that do and do not fail to rest: refractory period in the embryonic diapause of *Daphnia magna*

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In seasonal and temporary environments, Cladocera species depend on resting eggs that are able to survive harsh periods lethal to non-dormant individuals. Re-activation of resting eggs (RE) development is induced by environmental cues that signal the end of harsh season. However, freshly produced RE often remain refractory to environmental cues (refractory period) and gain sensitivity to the cues only after completion of this period. Refractory period is supposed to protect RE from fatal precocious termination of dormancy in response to untimely cues, such as occasional rains during dry season on winter thaws. Here we report hatching experiments on RE of *Daphnia magna* from different natural populations of contrasting climate zones. We also present data of on the parallel changes in embryo morphology, mRNA profile and re-activation ability during refractory period and after its completion. The results demonstrate strong intra- and inter-population variation of refractory period. While in some *D. magna* populations all RE have to pass weeks of refractory period before development resumption, in others up to half of produced eggs are able to develop almost without dormancy. This variation occurs both in summer-dry and winter-freeze habitats. Morphological studies and RNASeq data show that while no morphological changes or cell divisions occur during refractory period of dormancy, RE undergo dramatic changes in mRNA profile due to active gene expression or, alternatively, targeted RNA degradation probably involved in the control of diapause. Genes that are mass-expressed in *D. magna* RE include heat shock proteins, LEA proteins and trehalose pathway genes.

**Keywords:** resting eggs; dormancy; refractory period; local adaptation

# Comparative Transcriptomics of *Daphnia* and Bio-medical Models for the Evolutionary Conservation of a Gene Network for Fatty Liver Disease

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Genes that govern fundamental biological processes are deeply rooted in evolutionary history. Comparative evolutionary biology demonstrates that disease-associated genes are evolutionarily conserved across metazoans. The peroxisome proliferator-activated receptor α (PPARα) is a major transcription factor that regulates hepatic fatty acid and lipid metabolic processes in vertebrates. These metabolic processes can become perturbed by environmental compounds, leading to fatty liver disease, fibrosis and hepatocellular carcinoma. Environmental pollution is a global health crisis, and rodents have been the primary models for human chemical safety testing due to our shared mammalian biology. However, the importance of mammalian systems is being challenged by ethical and scientific considerations. Comparative transcriptomics facilitates our understanding of evolutionarily shared responses to toxicants and broadens the utility of alternative model species in predicting adverse human and environmental health. Despite the absence of a liver in invertebrates, the molecular networks governing liver fatty acid metabolism may be evolutionarily conserved and possibly predictive of adverse health in humans. Here we show that the microcrustacean *Daphnia* possesses amino-acid sequence orthologs of human genes for 70% of the PPARα signalling pathway. Moreover, *Daphnia* signals human pathways regulated by PPARα when exposed to a PPARα agonist. Additionally, we use comparative single nuclei transcriptomics of *Daphnia* and Zebrafish embryos to investigate the tissue-specific response to a PPARα agonist and to identify homologous liver-like tissues in *Daphnia*. This research shows promise for the reduction and replacement of mammals, and through conserved molecular biology, it proposes *Daphnia* as an alternative model for chemical safety testing of liver toxicity.

**Keywords:** evolutionary conservation; conserved molecular mechanisms; liver toxicity; single nuclei transcriptomics

# Genetic mechanisms of invasion success: A case study on *Cercopagis pengoi* in the Baltic Sea

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The evolutionary drivers behind the success of invasive species remain a puzzle, despite the significant ecological harm they inflict. Typically, successful invaders exhibit traits similar to r-selected populations, characterized by rapid growth and high reproduction, which facilitate their establishment in new environments. Consequently, the genetics of growth-related traits is crucial for understanding invasion biology. Earlier experimental studies on *Daphnia* implicate rDNA heterogeneity in the variability of somatic growth. These genetic elements play pivotal roles in ribosomal RNA production, protein synthesis, and growth, potentially contributing to the adaptability. To explore the variability of rDNA during the invasion, we investigated the onychopod cladoceran, *Cercopagis pengoi*, in the Baltic Sea since its introduction in 1994. We focused on intergenic spacer (IGS) and rDNA dosage variability in the populations at various stages of invasion. Also, these genetic traits were correlated to life-history traits, such as frequency of sexual reproduction and embryo development. Our findings suggest that shifts between parthenogenic and sexual reproduction evolve during dispersal, with newly established populations showing enhanced resting egg production and more rapid parthenogenic embryo development. Moreover, populations in initial colonization stages exhibited longer IGS, higher rDNA copy number, and higher RNA content than well-established populations. Similar to the earlier findings for *Daphnia*, we observed that the high-rDNA genotype was associated with high population growth capacity in *C. pengoi*. We propose that the inherent variability in rDNA architecture contributes to predisposing potential invaders to ecological success in novel environments. Understanding genetic adaptation mechanisms in biological invasions can inform a range of fields, from evolutionary biology and ecology to conservation and management.

**Keywords:** biological invasions; genetic adaptation; rDNA copy number, IGS length, facultative parthenogen

# Prevalence and differentiation of multiple defense types in *Ceriodaphnia cornuta* from lakes

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Evolutionary theory predicts that the evolutionary success of inducible defense needs predation risk fluctuation. Otherwise, we expect constitutive defense (including no defense) to evolve. Cladocerans, which contain diverse defense traits, are excellent model organisms for testing the evolutionary theory about inducible defense. In this study, we focused on the horns, a unique defensive trait in *Ceriodaphnia cornuta*, and tested the prevalence and differentiation of multiple defense types (no defense, inducible defense, and constitutive defense) in this species from natural lakes. We conducted whole-genome re-sequencing of 44 *C. cornuta* clones from 6 lakes and evaluated the horn expression of each clone under varying predation risks (control, fish kairomones, and *Chaoborus* kairomones). Our findings revealed the following: 1. There is remarkable differentiation of phylogenetic relationships among *C. cornuta* from different lakes; 2. All lakes harbor *C. cornuta* clones with multiple defense types, and their composition varies. Notably, in Nanyi Lake, where invertebrate predators are present most of the year, most *C. cornuta* clones perform constitutive defense; 3. Different types of defense types show strong differentiation at multiple functional genes, suggesting polygenic adaptation in the evolution of inducible defense. These results systematically demonstrate that the evolution of multiple defense types (including no defense, inducible defense, and constitutive defense) is prevalent in natural lakes and is unrelated to kinships, contributing to a deeper understanding of the evolutionary theory of inducible defenses.

**Keywords:** inducible defense; constitutive defense; kairomone; predation risk; genome sequencing

# Does the microbiome mediate priority effects in zooplankton community assembly?

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Priority effects, where the order of species arrival influences community assembly, are well-documented in zooplankton communities. For instance, strong priority effects have been demonstrated in competition trials between *Daphnia* species, the underlying mechanisms for which however remain unclear. In this study, we hypothesize that these effects are microbiome-mediated, with the first-arriving species conditioning their external environment with species-specific microbiota, which then serve to enhance their own fitness while inhibiting that of other species. To test this, we manipulated the immigration order of two *Daphnia* species, namely *D. magna* and *D. pulicaria* into 2L aquaria while maintaining a time-lag of two weeks. To dissect the role of the microbiome in strengthening/diluting these effects, water conditioned by the *Daphnia* species were collected from single species cultures and used as inoculum to supplement the priority effect treatments. To further dissect what component of the microbiome contributed to these effects, three types of inoculum from the conditioned medium were created: 1) unfiltered medium containing a combination of bacteria and their metabolites, 2) filtered medium retaining only metabolites, and 3) a resuspension medium comprising only bacteria of the conditioned medium. Although strong priority effects were observed, our results showed no significant effect of the microbiome in mediating priority effects between *D. magna and D. pulicaria*. These findings suggest that factors other than the microbiome may drive priority effects in these species, providing new insights into the mechanisms of zooplankton community assembly.

**Keywords:** (priority effects; community assembly; *Daphnia*; microbiome)

# Experimental contagious asexuality in *Daphnia pulex*

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Evolutionary transitions to asexuality are often studied by comparing asexuals found in nature with closely related sexuals. However, asexuals sampled in nature may represent only a small subset of the most successful lineages and may therefore contain limited information on the average properties of newly arising asexuals. Here, we generate new asexual lineages of *Daphnia pulex* by contagious asexuality, where rare males from obligate asexual lineages transmit asexuality to new lineages by cross-mating with sexual (cyclical parthenogenetic) females. We find that the newly created asexual lineages strongly differ from those sampled in nature, regarding their mode of asexual reproduction, loss of heterozygosity, recombination, and reproductive success. Together, these findings suggest that the generation of new, obligate asexual lineages through contagion may not be as straightforward as previously thought and may result in diverse, non-clonal offspring, on which subsequent selection may act. Our results also show the strength of laboratory-based experiments in complementing field-based studies, as the direction and degree of selection acting on novel asexual lineages can be inferred from the contrast between asexuals generated in the laboratory and older asexuals sampled in the field. The fact that similar results were obtained in a related, non-cladoceran species, *Artemia parthenogenetica*, suggests that the findings are general and may apply in many contagious asexuals, and perhaps even beyond.

**Keywords:** Cost of asexuality, *Daphnia pulex*, evolutionary sex-asex transitions, obligate parthenogenesis.

# Microplastic uptake by *Daphnia*: influence of temperature, food and oxygen availability

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Pollution of freshwater habitats with microplastics threatens filter-feeding zooplankton like *Daphnia*, which ingest microplastics while feeding, leading to their accumulation in the digestive tract. This study investigates the effects of food quantity, ambient temperature, and oxygen content on the uptake of 1 µm polystyrene beads by *Daphnia*. Under conditions of low food availability and increased temperature, the rate of microplastic uptake was elevated. This reflected the complex regulatory patterns of the water flow generated by the thoracic limbs: within one hour, an accumulation of microplastics in the organism was observed, which was 1160 times the concentration in the surrounding medium. Under hypoxia, microplastic uptake was low, indicating that *Daphnia* do not filter more under hypoxia and that sufficient oxygen reaches the cells through other adaptive mechanisms, such as compensatory tachycardia. The amount of energy reserves of *Daphnia* was used as an indicator of the metabolic status after microplastic uptake. A three-day microplastic exposure with and without feeding with *Desmodesmus subspicatus* had no impact on the lipid, carbohydrate and protein reserves beyond the response to the prevailing food and temperature conditions. Therefore, despite a digestive tract filled with microplastics, normal digestion and nutrient absorption were possible as long as food was available. Transferring the laboratory experiments to the habitat indicates that during the filter-feeding of zooplankton organisms in warm waters and under scarce food availability, as is the case during the clear-water phase of lakes in summer, an increased load of microplastic particles is to be expected.

**Keywords:** physiology; temperature; microplastics

# Effects of swimming biomechanics on prey recognition in *Daphnia longicephala*

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Daphnids form various defensive traits, e.g., inducible behavioural and morphological alterations in response to predators. These defensive traits have already been described in many species and were proven successful in predation experiments. However, the actual mechanism reducing the predation rate is mostly unknown, also in *D. longicephala*. This species is known to form an exuberant head extension protruding the back of the head to a crest-like structure in the presence of chemical cues of the backswimmer *Notonecta*. Furthermore, the larger, *Notonecta*-defended morph is known to swim faster. Some studies already described that defended morphs are attacked more often, but at the same time evade these attacks more efficiently. Therefore, we tested whether the turbulences elicited by the daphnids' locomotion play a role in *Notonecta's* detection and capture efficiency. We used particle image velocimetry (PIV) to visualize the flow fields around *D. longicephala* and the vortices left in the medium. That way we determined the volume of the medium affected by the daphnids locomotion movements. Moreover, we measured the velocity of the animals as well as of the fluid and evaluated the longevity of vortices. In addition to analysing swimming movements using PIV, we simulated water flow around the daphnids using computational fluid dynamics (CFD) to assess the increase of the defences' operational costs. Finally, we recorded predation experiments to verify the derived hypotheses. Based on these results we get deeper insights into how the predator-prey-interaction between *D. longicephala* and *Notonecta* is mediated by the biomechanical aspects of the defensive structures.

**Keywords:** biomechanics; streamline analysis; predator-prey-interaction; functional morphology; costs of defences

# Heat tolerance in the *Daphnia longispina* species complex

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To predict population, ecosystem, and evolutionary consequences of elevated temperature, it is crucial to identify and further investigate ecologically relevant traits in the context of global warming, especially in keystone species such as *Daphnia*. In the present study, we measured the heat tolerance as $CT_{max}$ (critical thermal maximum) and $T_{imm}$ (time until immobilization) for a set of clones from the *Daphnia longspina* species complex (DLSC), comprising the species *D. cucullata*, *D. galeata* and *D. longispina*. All clones were raised at 18°C and 22°C for at least two generations and fed *ad libitum* with the green algae *Tetradesmus obliquus*. $CT_{max}$ was measured along a temperature gradient (20°C to 45°C), while $T_{imm}$ was measured at a constant temperature of 34°C. We used video tracking to assess the swimming activity of the daphnids, and defined the timepoint at which they lost their motoric function using an algorithm based on rolling median and a defined activity threshold. We demonstrate that i) both $CT_{max}$ and $T_{imm}$ allow for the assessment of intra- and interspecific differences in heat tolerance between the clones, ii) heat tolerance in DLSC is higher in *Daphnia* acclimated to 22°C than 18°C, and iii) does not correlate with body size, iv) video tracking can be effectively used to measure heat tolerance in *Daphnia*. We suggest that heat tolerance is an ecologically relevant trait that plays a crucial role in shaping the community composition and driving evolutionary changes in *Daphnia* in response to elevated temperatures.

**Keywords:** global warming; traits; heat tolerance; intraspecific variability; hybridization

# Stress responses of polyploid Arctic *Daphnia* to elevated temperature: a resurrection approach

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Rapid environmental change poses challenges to organisms across the globe. Especially in the Arctic, environments are strongly impacted by recent changes in temperature, precipitation, and cloud cover. In these high latitudes, asexual polyploid *Daphnia* populations are common. We studied an asexual polyploid *Daphnia pulicaria* population in lake Braya Sø, South-West Greenland. Although asexuality is considered an evolutionary dead end, previous results suggest that this population has persisted as near genetically uniform for at least the last 200 years throughout very pronounced environmental changes. We used a resurrection ecology approach to compare members from a historical (11-year-old) and a modern *Daphnia* subpopulation. To test their phenotypic responses, two clones of each subpopulation were subjected to three temperatures (14 ºC, 16 ºC and 18 ºC) reflecting scenarios of future warming in the area. Life history traits as well as physiological stress markers were measured. The results suggest significant differences between clones both in fitness and plasticity. Investigating the responses of asexual clones from past and current time points of a single population can give us valuable insight into how this population manages to survive marked environmental change without the benefit of genetic recombination.

**Keywords:** asexual reproduction; physiology; life history; plasticity; adaptation

# Dimorphic regulation of male-determining gene *Doublesex1* expression in *Daphnia magna*

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Cladocera produce genetically identical males and females by parthenogenesis. A healthy population consists of parthenogenetic females. In contrast, parthenogenetic males appear only when the environmental changes such as shortened photoperiod, a lack of food and/or increased population density occur. To understand molecular mechanisms underlying environmental sex determination in Cladocera, we have examined the regulation of the male-determining gene *Doublesex1* (*Dsx1*) in *Daphnia magna*. During embryogenesis, *Dsx1* expression is upregulated in males and this male-biased expression is maintained throughout lifespan. Using genetic engineering tools including TALEN and CRISPR-Cas, we identified the three *Dsx1* regulators: bZIP transcription factor Vrille, RNA binding protein Shep, and long noncoding RNA *DAPALR*. Vrille is expressed in male embryos and upregulates *Dsx1* expression. Shep is expressed in both sexes, binds to *Dsx1* 5´ UTR, and represses its translation. *DAPALR* is transcribed from upstream of the *Dsx1* transcription start site and overlaps with *Dsx1* 5´ UTR. Therefore, Shep also binds *DAPALR*. Based on these findings involving Shep and DAPALR, we propose that Shep cancels the unexpected expression of *Doublesex1* and maintains the feminized state for sexual dimorphism, but *DAPALR* suppresses this repression by sequestration of Shep. This post-transcriptional regulation may function as a fail-safe system by which sexually dimorphic *Dsx1* expression is maintained, and sexual ambiguity is avoided. We infer this mechanism is not only for binary sex regulation but could function in the binary regulation of diverse phenotypic plasticity in Cladocera.

**Keywords**: *Daphnia magna*; environmental sex determination; male-determining gene; transcriptional regulation; post-transcriptional regulation

# Food quantity and quality modulate inducible defences in a common predator-prey system

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Zooplankton display different inducible defences against invertebrate and vertebrate predators. The response pattern to gape-limited invertebrate predators involves increased somatic growth and offspring body size but delayed maturity and reduced offspring numbers. In contrast to this general pattern, the freshwater model organism *Daphnia magna* has been reported to exhibit a different response when encountering the gape-limited tadpole shrimp *Triops cancriformis*. Under laboratory conditions, *D. magna* showed increased somatic growth, earlier maturation, and an increase in both offspring number and size. We propose here that the discrepancy between the previously observed and the theory-based response patterns against invertebrate predators is due to differences in food availability in the applied laboratory settings and assessed whether the defensive response of *D. magna* against *T. cancriformis* is modulated differently by food quantity and quality. We found a strong impact of food quantity and quality on the defence response of *D. magna* to *T. cancriformis* kairomones. The prey seems to be able to overcome trade-offs between morphological defence traits and reproductive traits, but distinctly between high food quantity and high food quality. Thereby, reproductive traits were preferred over morphological defences. Furthermore, removal of particles from the *T. cancriformis*-conditioned water caused a defence pattern in *D. magna* that was consistent with the general response pattern known from other invertebrate predators, thus explaining the described discrepancy to previous studies with *T. cancriformis*. Our study highlights the importance of assessing food-related effects on predator-prey interactions to understand trophic relationships and food web processes.

**Keywords:** *Daphnia*; food quality; food quantity; inducible defences; *Triops cancriformis*

# The response of the parasite *Metschnikowia bicuspidata* to elevated temperature in experimental evolution assay

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The hypothesis "warmer hence sicker world" states that global warming will result in increasing prevalence and virulence of parasitic diseases. However, these predictions often overlook the predisposition of parasites to evolve thermal adaptation rapidly. Elevated temperatures can enhance parasites performance against their host, due to their larger population sizes and shorter generation times. To investigate the adaptive response of parasites to elevated temperatures, we conducted an experimental evolution assay with a fungal parasite of *Daphnia magna – Metschnikowia bicuspidata*. Parasites were maintained at elevated (24°C) and control (20°C) temperatures over 6 parasite generations and the performance of each parasite line (5 lines per temperature) was subsequently assessed under both elevated and control temperature conditions. The evolutionary lines of parasites were further maintained over 20 generations under control and increased temperatures, and the experimental procedure was repeated with surviving lines. Reciprocal infection test revealed that in after 6 generations parasite fitness (prevalence and virulence) was diminished in lines evolving at higher temperatures across both test temperature conditions. While the single surviving line at 24 °C was able to increase its prevalence per *Daphnia* after 20 generations, the induced virulence remained on similar level compared to parasite lines kept at 20°C. These findings suggest that, contrary to expectations, the "sicker world" hypothesis may not hold true for the *Daphnia*-*Metschnikowia* system.

**Keywords:** *Daphnia*; host–parasite interactions; prevalence; fungal parasite; global warming

# Response to stress from different cyanobacteria species involves conserved sets of miRNA in *Daphnia magna*

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Differences in food quality can affect zooplankton fitness, especially in eutrophic water bodies where cyanobacteria are the dominant community. *Daphnia* is a direct grazer of algae due to its general grazing and is vulnerable to food quality. miRNAs, a novel class of small molecules that regulate gene expression at the post-transcriptional level, are conserved and may form the basis of species evolution. Therefore, based on the diversity of cyanobacterial bloom species, the importance of *Daphnia*-cyanobacterial bloom interaction and the effectiveness of miRNAs in regulating molecular responses, we carried out a conservation analysis of miRNAs under a variety of different cyanobacterial stresses using the model species, *Daphnia magna*. Differential miRNA and mRNA expression profiles of *D. magna* exposed to these five cyanobacteria showed that 49 differential miRNAs were conserved across treatments and that 30 of their target genes were also regulated and co-responsive. Functional annotation and enrichment analysis also revealed a high degree of overlap in metabolic processes such as pancreatic secretion, protein digestion and absorption. These results suggest that animal responses to food quality are nutritionally conserved and similarly regulated. Our results reveal molecular adaptations in zooplankton in response to different food qualities and elucidate the role of post-transcriptional modification in biological phenotypic plasticity as well as adaptive evolutionary patterns.

**Keywords:** *Daphnia*; harmful algal blooms; post-transcriptional modification; adaptative evolution

# From eco-to-evo to evo-to-eco with the *Daphnia longispina* species complex

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Rapid global change comprises multiple ecological changes affecting niche space and distributional ranges of species and, thus, also reproductive isolation among closely related species. Consequently, rapid ecological change can result in almost instant evolutionary change by favoring the generation of hybrids and backcrosses. Such rapid ecological change leading to rapid evolutionary change is also known as the eco-to-evo component within the framework of eco-evolutionary dynamics. Such evolutionary changes, in turn, have the potential to drive ecological change - corresponding to the evo-to-eco component of eco-evolutionary dynamics - which can result in a completed eco-evolutionary feedback loop. Here, I first summarize recent work using whole genomes from resting egg banks of Lake Constance, Lake Zurich, Lake Annecy and Lake Varese contributing to a more detailed understanding of the consequences of rapid ecological changes on taxon turn-over and hybridization dynamics in the *Daphnia longispina-galeata-cucullata* complex. Then, I will report on a large-scale survey of > 40 peri-Alpine lakes and our attempts to learn more about the effects of several decades of environmental change on the composition and evolution of the *Daphnia* communities in these lakes. Finally, I will present a preview of ongoing and planned work to characterize the evo-to eco leg of a putative eco-evolutionary feedback loop featuring the *D. longispina* complex in peri-Alpine lakes.

**Keywords:** *Daphnia longispina* complex; hybridization; global change; eco-evolutionary dynamics

# Elevated temperatures impact *Daphnia* fitness, but not its resistance to a fungal parasite

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Increasing lake temperatures, associated with global warming, are changing the structure of aquatic communities and may also increase the susceptibility of *Daphnia* species to parasitism. We tested how the fitness and resistance of *Daphnia* is impacted by exposure to increased temperatures across short and long-term periods. Our study site contains lakes that have been artificially heated by the output of a nearby power plant for over 60 years. These lakes are on average ~4°C hotter than the surrounding unheated lakes. We sampled 12 *Daphnia* individuals from two heated and two unheated control lakes and kept clonal replicates in incubators set to 18 °C (control) and 22 °C (elevated). We exposed these experimental clones to *Metschnikowia* spores to test resistance to parasitism. We hypothesised that *Daphnia* isolated from artificially heated lakes would be more susceptible to parasitic infections. Our results showed very low levels of infection (< 7%) and that there was no significant impact of exposure to *Metschnikowia* spores on our *Daphnia* fitness. Our results also showed that *Daphnia* from heated lakes had significantly lower fitness (when kept at 18°C) compared to *Daphnia* from unheated lakes. However, when these same *Daphnia* clones (from heated lakes) were kept at 22°C, they were shown to have significantly higher fitness. These results could indicate that there is a greater effect of temperature (short-term & long-term) on *Daphnia* fitness than its influence on resistance to parasites.

**Keywords:** *Daphnia*; limnology; parasites; temperature; evolution

# How was the genetic structure of *Daphnia pulex* population in a small lake developed from the early colonization? Examination by paleolimnology, laboratory experiment and theoretical analysis

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How biological populations colonize and establish in novel habitats is a central question in ecology. Although Population genetic diversity and structure affect it, our understanding of actual population genetic changes from the early colonization stage is limited. In this study, using a paleolimnologcal approach with diapausing eggs, we revealed the long-term dynamics of an obligated parthenogenetic panarctic *Daphnia pulex* population during the early stage of colonization and subsequent establishment in Lake Fukami-ike, Nagano, Japan. We found *D. pulex* population was colonized and maintained by one predominated haplotype, and was limited genetic diversity. In recent years, two haplotypes that were genetically distant from a predominated haplotype appeared but did not replace the predominated one. We also examined the mechanism that promoted coexistence of a predominated one and later established one with laboratory experiments and theoretical analyses. We focused on diapause induction. We established monoclonal cultures of the two distant haplotypes from ephippia of them preserved in the layers of sediment core samples in which the two haplotypes coexisted. Laboratory experiments showed one haplotype tended to produce diapausing eggs in broader environmental conditions than the other. Empirically parameterized theoretical analyses suggested that different photoperiodic responses can promote coexistence via the storage effect with fluctuations of the growing season length. Throughout the study, we suggest that an obligate parthenogenetic population can establish in a new habitat and be maintained for generations even though it has limited genetic diversity; and the timing of diapause induction might promote coexistence of two genetically distant haplotypes.

**Keywords:** *Daphnia pulex*; paleolimnology; population genetic dynamics; storage effect; diapausing

# Genetic variation in rapid adaptation to the invasion of a predator in a natural *Daphnia magna* population

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Predation is a key factor shaping ecosystems, and the invasion of a fish predator can severely impact freshwater habitats by altering zooplankton communities. *Daphnia* are famous for showing several defence strategies when facing the fish predator, including morphological, life-history and behavioral changes. The selection pressure by predation on *Daphnia* populations is typically strong and phenotypic changes in response to changes in predator regime rapid, and often include complex phenotypic responses. The underlying genetic architecture has been described as polygenic and several hundreds of single nucleotide polymorphisms (SNPs) were detected to be under selection in adaptation to fish predation. Here, we study a recent fish invasion to a previously fish-free gravel pit lake in Northern Germany and its consequences for the local *Daphnia magna* population. After its establishment in 2006, the lake harbored a rich zooplankton community including *Daphnia magna* and *Daphnia pulex*. In 2019, first individuals of the fish species common sunbleak (*Leucaspius delineatus*) were detected and the fish population increased massively over the following years. We analyzed the consequences for the zooplankton community in general and, more specifically, the genomic and phenotypic characteristics and changes in the local *D. magna* population. We further compare our results to predictions made from population genetic simulations mimicking the scenario of fish invasion as well as to data from other European *D. magna* populations experiencing selective pressure by fish predation.

**Keywords:** genomics; predator-prey interaction; natural population; fish predation; rapid adaptation

# Can *Daphnia* teach us how to live longer without eating less?

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*Daphnia* was one of the first organisms in which the life-extending effect of caloric restriction (CR) has been demonstrated and there is ample evidence that non-starving low food levels extend lifespan by 40-50%. Mimicking CR in model organisms without actually restricting caloric intake is intriguing due to potential translational or “biohacking” implications. We tested two compounds often hypothesized as possible life extension intervention agents: b-hydroxybutyrate (BHB), a ketone body, and nicotinamide mononucleotide (NMN), a rate-limiting NAD+ precursor, in a common-garden experiment with two *Daphnia magna* genotypes kept on *ad libitum* food, with a CR treatment as a comparison. Specifically, BHB supplementation is expected to activate FOXO transcription factors thus upregulating genes involved in antioxidant pathways and regulate glucose metabolism. NMN supplementation has been shown to slow down age-related decrease in NAD+ and NAD+/NADH ratio, a key indicator of redox health and to improve mitochondrial function and reduce inflammation. We show that BHB extends life by reducing young individuals mortality to the CR level. NMN did not have a similar effect and slightly diminished the effect of BHB in a double-chemical treatment. We discuss possible genotype-by-intervention interactions and transcriptome differences between *ad libitum* food control, CR, and the BHB and NMN treatments.

**Keywords:** *Daphnia*; longevity; b-hydroxybutyrate; nicotinamide mononucleotide; caloric restriction

# A non-monophyly of “crowned” *Daphnia* from the Palaearctic: multiple losses or gains of the defence against tadpole shrimps?

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Various clades of *Daphnia* exhibit a wide range of phenotypically plastic antipredator defences, such as helmets and crests. Several taxa within the subgenus *Ctenodaphnia* exhibit a head plate, a projection of carapace into the head shield, which may be adorned by a prominent spinulation nicknamed “crown of thorns”. We have demonstrated that this structure is phenotypically plastic within the Eastern Palaearctic *Daphnia atkinsoni* complex and serves as an antipredator adaptation against tadpole shrimps (Notostraca). However, additional “crowned” *Daphnia* have been described from other biogeographic regions, including *D. triquetra* from Central Asia, *D. coronata* from South Africa, and *D. crassispina* from Greenland. We aimed to evaluate the monophyly of Eurasian “crowned” *Daphnia* based on genomic phylogenies and morphology, including representatives of two clades of the *D. atkinsoni* complex and *D. triquetra*. Both whole-mitogenome and partial nuclear phylogenies (based on >680 single-copy genes) clearly rejected our hypothesis of the monophyly of the Palaearctic “crowned” daphnids, as other species lacking the head plate were interspersed between the *D. atkinsoni* complex and *D. triquetra*. Laboratory experiments with *Triops* kairomones nevertheless confirmed that the crown in *D. triquetra* can also be induced by this chemical stimulus. In contrast, clones of a “crown-less” *D. atkinsoni* clade from the Golan Heights, which apparently does not encounter tadpole shrimps in its habitats, did not respond to *Triops* kairomones. This suggests that the ability to form the “crown” either developed or – more likely – got lost multiple times during evolution of this *Ctenodaphnia* clade.

**Keywords:** inducible defences; crown of thorns; *Triops*

# Zooplankton of ephemeral wetlands on arable land: unique communities influenced by landscape history

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The intensification of agriculture is one of the greatest threats to biodiversity. For example, 40% of the European landscape has been converted to arable fields, where ephemeral wetlands present small islands of biodiversity surrounded by large areas of agricultural landscape. Their ephemeral character in combination with the absence of fish shapes zooplankton communities in two ways. First, they provide a habitat for species specialised in ephemeral habitats. Second, they host species susceptible to fish predation. Unlike fish, many zooplankton species can survive adverse conditions in resting eggs, or resting stages in the sediment. Planktonic crustaceans thus form seed banks that can persist in the sediment for decades and may reflect the history of the site. Between 2015–2022, we collected 106 samples from 48 field ephemeral wetlands in southern Moravia (Czech Republic). We found that sites that were wetlands in the mid-19th century host more species-rich communities today than sites without such historical continuity supporting also the occurrence of several regionally rare species (e.g. *Daphnia atkinsoni*, *Wlassicsia pannonica*). In addition, zooplankton communities clearly differed between the spring and summer samples, which is probably related to temperature variation and the effect of the seed bank. This was also reflected in the fact that the variability of communities at repeatedly sampled sites did not differ from that between sites. We conclude that ephemeral wetlands on arable land should be protected as they differ from other lentic biotopes due to the absence of fish, thus providing refuge for rare zooplankton communities.

**Keywords:** diversity; assemblage; wetland, arable field; habitat history; seed bank

# *Daphnia* as a living sensor for environmental monitoring

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Traditional water monitoring methods typically involve sampling or using commercially available sensors. While reliable, these approaches have limitations in terms of speed and/or robustness. A novel approach utilizing living organisms offers continuous monitoring by observing their reactions to environmental changes. *Daphnia magna*, chosen for its sensitivity and unique stress behaviours, was chosen as a reliable bioindicator for this purpose. Through automated image analysis, their behaviours provide insights into water quality. A field setup designed for observing their stress reactions includes a flow-through cage monitored by a Raspberry Pi camera, enabling continuous registering of the behavioural characteristics and their on-board analysis. The use of low-power electronics for continuous data collection extends the duration of monitoring missions significantly. Calibration of this novel sensor type involves testing the animals against various toxins under laboratory conditions, focusing on apparent stress reactions, including "spinning" behaviour or movement inhibition. Several stressors, such as increasing salinity, the Diesel water-soluble fraction, and various herbicides, are being tested or in the testing phase. Preliminary experiments have demonstrated *Daphnia*'s potential as a sensor for biohybrid monitoring systems, thanks to its immediate and clearly readable reactions. This approach facilitates the early detection of toxins, diseases, and invasive species, as well as changing abiotic parameters (pH, temperature etc.). It aids in the mitigation of water quality issues in aquaculture systems and protected natural areas. Its cost-effectiveness and ease of application make it an appealing tool for sustainable environmental management.

**Keywords:** biohybrids; *Daphnia*; toxicity; environmental monitoring; animal-robot interaction

# Mineral oil, movement and monitoring ecosystem health

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Commercial formulations of mineral oils and other forms of lubricants can be released into the environment during the application, use and lifetime of a product. Mineral oil formulations in combination with other materials such as graphene and silicone nanomaterials can be used to prolong the life and use of sporting equipment, such as bikes and boats. Through this, excess material can be released into the environment during application. Oil is a challenging pollutant to monitor and assess in aquatic environments due to the hydrophobic nature which makes the distribution and movement of oil different to other types of aquatic pollutants. Here, we explored the impacts that commercial formulations of mineral oil (with and without graphene/silicon) can have on the keystone freshwater species *Daphnia magna. Daphnia* were exposed to different formulations and concentrations of mineral oil and their acute toxicity was initially assessed, before subsequent studies were undertaken to determine how oil can impact swimming behaviour. This was done by recording movement over a 24-hour period and assessing vertical migration in a cuvette and spins/movement in a 10mL well for stress indicators (i.e. more spins in the water are behavioural indicators of higher stress). Through evaluating *Daphnia* movement and interaction with the oil in different test vessels we can also explore how they might influence the transport and mixing of oil into their environment and impact the legacy timeframe of oil contamination.

**Keywords:** oil; swimming; behavioural toxicity; movement; advanced materials

# Exploring linkages between dysbiosis and host responses in *Daphnia magna*

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The microbiome plays a critical role in multicellular organisms by providing essential functions to its host. Environmental disturbances can interfere with these processes by negatively affecting the microbiome´s diversity and composition, a phenomenon known as dysbiosis. However, despite the increase in microbiome research over the past decade, our understanding of the mechanisms and consequences of shifts within the microbiome community due to chemical stressors remains limited, and the information gained is often insufficient to use in an (eco)toxicological content. In this project, we used non-caloric artificial sweeteners (AS) as potent microbiome modulators to investigate microbiome responses in the model organism *Daphnia magna* and its downstream effects on the host´s physiology, behavior, and life-history traits. The project aims to (I) evaluate whether exposure to AS can alter the microbiome and cause dysbiosis in daphnids, and (II) improve the mechanistic understanding of AS effects with a particular focus on the microbiome's role in the catecholamine pathways of *Daphnia* suggested by earlier studies in vertebrate and invertebrate species, and (III) its downstream effects on animal swimming behavior, cardiovascular performance, feeding, and growth.

**Keywords:** *Daphnia magna*; microbiome; disbiosys; artificial sweeteners

# The cladoceran (Crustacea, Branchiopoda) diversity in the floodplains of Tonle Sap Lake and Mekong River in Cambodia

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The investigation of cladocerans in Cambodia, a region known for its high biodiversity in Southeast Asia, has received limited attention. The Tonle Sap River connects Tonle Sap Lake to the Mekong River in the vicinity of Phnom Penh. Due to the flooding of the Mekong, water backs up into the Tonle Sap River and flows into Tonle Sap Lake. The dry-season lake, which has a length of 120 km and a width of 35 km, undergoes flooding and reverse floods that transform it into a wet-season lake with dimensions of about twice in size. One hundred and fifty samples from 147 freshwater habitats in the floodplains of Tonle Sap Lake and the Mekong River in Cambodia were collected using a 60-meter mesh-sized plankton net during the pre-monsoon and post-monsoon seasons. We recorded a total of 50 cladoceran species from 36 genera, 17 of which are new to Cambodia. *Diaphanosoma excisum* was found at 44.9% of the sites that were sampled. It was followed by *Ephemeroporus barroisi* (44.2%), *Ceriodaphnia cornuta* (39.6%), *Moina micrura* (37.3%), and *Chydorus eurynotus* (29.9%). Rare species were *Alona affinis*, *Chydorus parvus*, *Coronatella rectangula*, *Disparalona hamata*, *Dunhevedia crassa*, and *Grimaldina brazzai*. In addition, *Kurzia brevilabris*, *Pseudochydorus globosus, and Macrothrix pholpunthini* were extremely rare species. The fauna, typical of Southeast Asia or the Oriental region, bears striking resemblances to that of neighbouring countries, particularly Thailand and Laos. It is necessary to discuss the species' present taxonomic status, as these are preliminary findings.

**Keywords:** biodiversity; Cladocera; freshwater habitats; Southeast Asia; taxonomy

# Off-host parasite stages in a climate-changed world: Implications for host-parasite interactions

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Host-parasite interactions are an integral part of our ecosystem. Hence, it is essential to understand how environmental factors, such as heat waves and dryness, influence these interactions. While it is widely acknowledged that elevated temperatures strongly impact infection-related traits of both the host and its parasites, little is known about the direct effects of environmental factors on off-host parasite stages. We used the parasitic bacterium *Pasteuria ramosa* to examine how temperature variations during the off-host stage can affect the infection dynamics of its host, the water flea *Daphnia magna*. We exposed *P. ramosa* spores to various combinations of temperatures and exposure durations, ranging from ambient (20°C) to elevated temperatures (up to 60°C) in increments of 5°C (total of 8 temperatures), with exposures ranging from 1 to 21 days, (total of 7 durations). Thereafter, we exposed healthy *Daphnia* to each of these combinations, and recorded infection status, parasite-induced host mortality (virulence), and parasite spore production (parasite fitness). Our results suggest that both temperature and exposure duration influence infection dynamics. On a broader scale, this study emphasizes the importance of considering the direct effects of climate change on off-host parasite stages, to better understand disease dynamics in a climate-changed world.

**Keywords:** *Daphnia magna*; *Pasteuria ramosa*; Off-host parasite stage; Temperature; Climate change.

# The dynamics of cladoceran invasions in pelagic food webs: a comparative analyses of the invasions of *Diaphanosoma brachyurum* and *Daphnia cucullata* into oligotrophic Lake Constance

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The cladoceran community of Lake Constance has been studied since more than one century, during which the lake experienced severe eutrophication and subsequent oligotrophication. During the last 2 decades two species were able to successfully build up a new persistent population in the lake: *Diaphanosoma brachyurum* from 2001 onwards*, and Daphnia cucullata* from 2015 onwards. Both species, however, differ regrading previous occurrences in the lake: while *D. brachyurum* was a stable member of the plankton community until the eutrophic period, when it disappeared from the lake*; D. cucullata* did never establish a persistent population during the last century prior to 2015. While the reason for the re-invasion of *D. brachyurum* is unknown, the invasion of *D. cucullata* seems to be linked to increased fish predation pressure due to the invasion of sticklebacks in the pelagic zone of the lake. This contribution compares the phenologies, population dynamics, and the seasonal spread of both cladoceran species and examines how long it took the two species to reach carrying capacity in the new environment.

**Keywords:** invasions; phenology; oligotrophication; fish predation

# Colonization success and evolution of *Daphnia* cf. *pulex* in a continental archipelago

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*Daphnia pulex* was once considered a cosmopolitan species, but molecular phylogenetic studies have shown that it is a complex species composed of at least eight genetically distinct species. One of these species, panarctic *D. pulex* (hereafter, *D*. cf. *pulex*), is widely distributed in North America. Although *Daphnia* species reproduce parthenogenetically under favorable conditions and switch to sexual reproduction to produce resting eggs, some lineages of *D*. cf. *pulex* produce resting eggs asexually and are therefore obligate parthenogenetic populations. Recent studies have shown that these lineages have expanded their range from North America to other continents and continental islands, including the Japanese archipelago. Field surveys in Japan have shown that *D*. cf. *pulex* is widely distributed in small lakes throughout the country. They are all obligately parthenogenetic and consist of four phylogenetically distant lineages JPN1~4, suggesting that four genetically distinct genotypes were the founders of the Japanese populations. Among these, JPN1, which is widely distributed throughout Japan, and JPN2, which is distributed in eastern Japan, showed multiple genotypes within the lineages. If these genotypes had evolved in Japan, JPN1 and JPN2 would have invaded Japan about 400 and 300 (±100) years ago, respectively. On the other hand, the JPN3 and JPN4 lineages have a limited range and few genotypes, suggesting that they have recently invaded Japan. In this talk, I will review recent studies on the ecology and evolution of *D*. cf. *pulex* invading the Japanese archipelago.

**Keywords:** niche difference; invasion success; competition; trait variation; nearly neutral theory

# The Evolutionary History of the Cladocera: Recent Insights and Future Perspectives

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The waterflea Tree of Life has fascinated researchers for centuries. In particular the phylogenetic relationships between the cladoceran families and orders, as well as the timing of radiations, have remained elusive. Thanks to the increased knowledge about the group and several fascinating techniques that have emerged in the last years, some of the most obscure branches of the Cladocera Tree are now being illuminated with a fresh light. The new insights can provide important clues on important sister lineages and how we interpret the phenotypic evolution (a lot of convergence) and timing of radiations in the group. Yet the new insights can also provide a new framework for particular aspects, such as the evolution of gene families of interest. I will highlight the latest insights in cladoceran evolution, based on 25 years of studying the group's morphology, diversity and paleontology, as well as phylogenomics (phylogenetics using entire genomes) and comparative genomics. The Tree is finally perhaps bearing fresh fruits (recent insights), and now we can figure out how to use them in brand new recipes (future perspectives). This talk is dedicated to those cladocerologists passionate about cladoceran evolution (Ebert, Kotov, Dumont, and many others), who are an integral and highly inspiring part of these studies, most crucial to our current understanding of cladoceran evolution.

**Keywords:** evolution; phylogenetics; phylogenomics; morphology; convergence

# Response of zooplankton community with varying evolutionary potential to stickleback predation

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Fish predation is known to be a strong selective pressure on zooplankton communities in freshwater systems and anti-predator responses in *Daphnia* genus has been extensively studied. We aimed to deepen understanding of how zooplankton communities respond to fish predation and to what extent genetic variation drive these responses. To that end, we compared two prey communities with varied genetic diversity in response to the presence/absence of fish predation. The zooplankton community consisted of three *Daphnia* species (*D. magna*, *D. pulicaria* and *D. galeata*), equivalent in starting biomass, but different in the amount of genotypes per species that was inoculated (10 genotypes vs 1 genotype). These communities were exposed to fish predation or no predation in outdoor mesocosms. We tracked the community assembly and phytoplankton concentration over time for seven weeks. We found that the change in community composition over time differed depending on predation regime and on the amount of genetic diversity. High diversity led to less variation in responses, while low diversity led to more distinct trajectories over time. Furthermore, a strong effect of zooplankton community on the algal concentration was observed as it caused significant differences in phytoplankton concentration between predation regimes & between diversity treatments within the mesocosms with fish present. Using a multi-species approach allows us to compare the anti-predator responses of *Daphnia* species and investigate if differences are related to their body size. Additionally, it yields us the opportunity to quantify if genetically diverse communities are better equipped to handle predative stress.

**Keywords:** *Daphnia*; Eco-Evo dynamics; Multispecies; Fish predation

# Effects of invasive fish on high mountain lake Cladocera and their recovery after fish removal

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High mountain lakes are originally fishless, although many have had introductions of non-native fish species, predominantly trout, and recently also minnows introduced by fishermen that use them as live bait. The extent of these introductions is general and substantial often involving many lakes over mountain ranges. Predation on native fauna by introduced fish involves profound ecological changes since fish occupy a higher trophic level that was previously inexistent. In this study we have studied the effects of introduced fish on high mountain lake Cladocera from the Alps and the Pyrenees and their recovery after fish removal. We have found that lakes with only minnows, have the strongest effect on *Daphnia,* followed by brook trout, while rainbow and brown trout had no significant effect. In the littoral, the factors explaining species distribution were fish abundance and macrophyte cover. In the littoral, the presence of fish favored a greater abundance of chydorids, while minnows also excluded the planktonic or macrophyte associated species such as *Simocephalus vetulus* and *Scapholeberis mucronata*. With the context of three conservation projects, LIFE BIOAQUAE (2012-2017), LIFE LIMNOPIRINEUS (2014-2019) and LIFE RESQUE ALPYR (2022-2026) we have removed or are in the process of removing both trout and minnows from 32 high mountain lakes of the Catalan Pyrenees and the Italian Alps. We have found that *Daphnia* recovered in most lakes where they were absent, and in the littoral, minnow eradication was accompanied by an increase in species richness as well as the appearance of planktonic and macrophyte associated species.

**Keywords:** *Daphnia*; high mountain lakes; Cladocera; trout; minnows

# High concentration of small nanoplastics increases diversity and shifts the microbiome composition of *Daphnia* in both parasite-infected and uninfected hosts

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Nanoplastics (NP) as emerging pollutants raise concerns due to their potential adverse effects on the aquatic environment. While toxicity studies demonstrate NP's impacts on aquatic organisms such as zooplankton, effects on ecological interactions, including host-microbiome and host-parasite systems, which are critical for assessing environmental health, are often overlooked. Here, we investigate the effects of NP and parasite infection on the diversity and composition of the host-associated microbiome in the model organism *Daphnia magna*. In a full factorial design, we exposed *D. magna* to: two NP sizes (50 nm and 100 nm), at two concentrations (low - 1 mg/L and high - 5 mg/L) and, with and without parasite infection by the fungal yeast parasite *Metschnikowia bicuspidata*. Microbiome profiling of *D. magna* gut and body tissues was conducted using 16S rRNA gene sequencing. Exposure to a high concentration of 50 nm NP shifted bacterial abundances with increase in the Orders Caulobacteriales and Rhizobiales, and decrease in the Order Chitinophagales. Diversity and richness of gut and body microbiomes increased after exposure to high concentration of 50 nm NP. Bray-Curtis dissimilarity measure of bacterial composition revealed significantly different bacterial community compositions in gut and body microbiomes irrespective of parasite infection, after exposure to high concentration of 50 nm NP. Alpha and beta diversity measures showed that the interactive effect of NP size and concentration have a stronger influence in shaping the *Daphnia* microbiome than parasite infection. These insights are crucial for understanding the consequences of NP pollution on ecological interactions in aquatic ecosystems.

**Keywords:** host-microbiome; *Metschnikowia bicuspidata*; 16S metabarcoding; microbiome

# Induction of diel vertical migration in *Daphnia* by a bile salt from fish: a mesocosm experiment, in-situ concentrations and threshold concentrations

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Diel vertical migration (DVM) is the largest synchronous movement of biomass on earth. In case of *Daphnia*, DVM is induced by infochemicals released by fish. Only recently, a first DVM-inducing infochemical from fish has been identified as the bile salt 5-alpha-cyprinolsulfate (CPS). This identification was based on *Daphnia magna* as the test species and an indoor DVM bioassay, in which several vertical gradients differed from those in stratified lakes. We here aimed to validate the role of CPS for DVM-induction in a more natural setting and performed mesocosm experiments using a natural zooplankton community. We could show that upon addition of CPS the vertical zooplankton distributions changed in accordance with the onset of DVM. This was particularly the case for *Daphnia longispina* indicating that the triggering of DVM by CPS is not limited to one *Daphnia* species. In the indoor bioassay the threshold concentration of CPS for induction of DVM was 0.1 nM. In order to investigate how generally valid this value is, we have determined threshold concentration of CPS for the induction of DVM in small-bodied *D. longispina* within mesocosms and through indoor bioassays, while also measuring in-situ concentrations of CPS across one season. These data will allow insights into spatial and seasonal variations of in-situ CPS concentrations in a lake and relate these concentrations to threshold concentrations for induction of DVM. More generally, these data will for the first time allow to sketch out a landscape of fear for *Daphnia*.

**Keywords:** DVM; *Daphnia*; infochemical; chemical communication

# Application of genome editing technique on *Daphnia magna*

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While the use of aquatic organisms to assess water environments has a long history, advancements in technologies such as genome editing have enabled a deeper understanding and application at the genetic level in recent years. Here, we report on the application of genome editing techniques to microcrustaceans used as environmental indicator organisms in aquatic environments, *Daphnia magna*. *Daphnia magna* is commonly used in environmental toxicity tests due to its sensitivity to chemicals, rapid growth, reproduction, and ease of cultivation. By developing genome editing techniques for *Daphnia magna*, we have been able to create a system for monitoring water environments more conveniently. One is the development of indicator organisms that visualize environmental responses. By introducing a gene encoding a fluorescent protein with the regulatory regions of environmentally responsive genes, we have succeeded to create strains that emit fluorescence in response to heavy metals or hormone-like chemicals, enabling real-time monitoring of changes in water environments. We are also using genome editing techniques to prove the mechanisms of environmental response. For example, when the phase I p450 gene is disrupted by genome editing, sensitivity to specific chemicals increases, revealing the gene that is actually involved in toxin metabolism in *Daphnia magna*. These approaches are shedding light on the mechanisms underlying environmental responses including sensitivity to chemicals.

**Keywords:** genome editing; *Daphnia magna*; heavy metal

# Using Resurrection Ecology to Study Environmental Change: Genes, Mud and Water Fleas

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Many types of organisms ranging from microbes to plants to metazoans like cladocerans produce diapausing or “resting” life-stages (e.g., cysts, spores, seeds, embryos/eggs). Among the Cladocera, much work over the past several decades has focused on members of the Genus *Daphnia* as a model for the burgeoning field of “Resurrection Ecology” (RE). In my talk today, I will present an overview of a variety of eco-evolutionary genetic studies that have taken advantage of resurrecting “resting eggs” (a.k.a. ephippia) of various cladoceran species from lake/pond sediments. Together, we will examine how populations are responding (i.e., evolving, or not) to environmental changes driven by a variety of biotic and abiotic stressors. Many of these stressors have anthropogenic undercurrents such as introduction of invasive species, alterations to physico-chemical features of aquatic habitats, and climate-change driven factors. I will conclude with talking about how the use of a RE-approach, coupled with developments in population genetics/genomics and ecological monitoring place members of the Cladocera (with a particular emphasis on *Daphnia*) in the vanguard of model organisms for understanding eco-evolutionary dynamics in aquatic systems.

**Keywords:** *Daphnia*; eco-evolutionary changes; resurrection ecology

# Sensing predation - mechanisms underlying predator perception in the freshwater crustacean *Daphnia*

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Ecological communities are organized in trophic levels sharing manifold interactions forming complex food webs. Infochemicals have a significant impact on these interactions, e.g. by inducing defenses in prey. The micro-crustacean *Daphnia* is able to respond to predator-specific chemical cues indicating an increased predation risk. *Daphnia* shows plastic responses by adapting its morphology, behavior and physiology, increasing organism and population fitness. We studied the molecular mechanisms of predator detection and elucidate the involved chemoreceptors, in line with the neuronal signaling cascades and the cellular adaptations in the vicinity of morphological defense expression. Using RNAi, we determined the involvement of two chemo-co-receptors involved in chemoperception. With the help of immunohistochemistry, confocal imaging and morphometrics, we found neuronal plasticity in line with a rewiring of cell-cell connections in the central brain. With the help of an EdU cell proliferation assay, we determined that a substantial increase in cellular size finally leads to the formation of defensive morphological features. Our data contribute to the understanding of the sensory pathway involved in predator perception. Future work will address, predator specific tuning chemoreceptors and further detail the neurotransmitter networks engaged in odor coding.

**Keywords**: chemoreception; ionotropic receptor; differential gene expression, RNA interference)

# A step towards understanding ecological impacts of artificial sweeteners using *Daphnia magna*

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Artificial sweeteners (AS), such as acesulfame and sucralose, are common additives used in food products. These sweeteners are considered emerging contaminants as they have low or no degradability in sewage treatment plants, indicating that they can impact the environment. Using *Daphnia magna* as a model species, we studied ecologically relevant impacts of AS, including neurotoxicity (acetylcholinesterase activity, AChE), oxidative status (lipid peroxidation, TBARS, and oxygen radical absorbing capacity, ORAC), and cardio-locomotor activities (heart rate and swimming). At environmentally relevant acesulfame and sucralose concentrations, we found dose-dependent responses for all markers and activities, suggesting a high potential for reduced daphnid fitness in field conditions when AS is present in the water. Moreover, the AChE response was linked to both oxidative biomarkers, with positive and negative relationships for TBARS and ORAC, respectively, indicating the need to understand these responses in concert. This work now serves as the starting point for a new series of experiments to study the mechanisms of action for AS, with a primary focus on host-microbiome interactions and their links to adverse effects via alterations of the host physiology and behaviour.

**Keywords:** *Daphnia magna*; behaviour; neurotoxicity; oxidative status; artificial sweetener

# Automated *Daphnia* measurements for phenotypic plasticity research

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Cladocerans of the genus *Daphnia* are ecological keystone species, and are often studied for their phenotypic plasticity. Measurements of *Daphnia* body parts are carried out in laboratories all over the world in time-consuming and expensive experiments. To enable faster and more reliable measurements we developed an open workflow using Fast Region-based Convolutional Network (Fast-RCNN) algorithms, which automatically recognise body parts and measure body dimensions. Reliable detection of *Daphnia* body parts in test-datasets including low quality images allows for automated measurements of body regions with human precision but without observer bias. Additionally, we were able to utilize a standard convolutional neural network (CNN) to classify species. Our current model classifies pictures of *Daphnia magna, D. pulex, D. longicephala, D. longispina,* and *D. cucullata* with accuracy of 94%. Automated recognition of the heart region in combination with frequency amplification allows for automated measurements of *Daphnia* heart rates. Explainable AI will allow for recognition of morphological reactions of potentially unknown traits to kairomones and sublethal stressors. Our model and algorithms are open source, available and adaptable for other research questions.

**Keywords:** *Daphnia*; life history; body size; classification; heart rate; observer bias

# The origin of obligate parthenogenesis in *Daphnia*

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Investigating the origin of parthenogenesis through interspecific hybridization can provide insight into how meiosis may be altered by genetic incompatibilities, which is fundamental for our understanding of the formation of reproductive barriers. Yet the genetic mechanisms giving rise to obligate parthenogenesis in eukaryotes remain understudied. In the microcrustacean *Daphnia pulex* species complex, obligately parthenogenetic (OP) isolates emerged as backcrosses of two cyclically parthenogenetic parental species, *D. pulex* and *D. pulicaria*, two closely related but ecologically distinct species. In this talk, I will focus on our recent progress of using transcriptomics to understand the genetic modifications and genetic incompatibilities that lead to the origin of obligately parthenogenetic *Daphnia*. Our transcriptomics data show that the underexpression of key meiosis genes is critical to the origin of obligate parthenogenesis. Our data also strongly support the idea that obligate parthenogenesis originated through the extension of existing parthenogenetic cell division in the ancestral cyclical parthenogenesis to resting egg production in OP hybrids, which is likely caused by genetic incompatibilities in the parental species. Lastly, these transcriptomic data provide important candidate genes for functional analysis to advance our understanding of the genetic basis of obligate parthenogenesis.

**Keywords:** introgression; underexpression; genetic incompatibility; meiosis; parthenogenesis

# Genomic adaptation of *Daphnia cucullata* to elevated water temperatures: Insights from power plant-heated lakes

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Freshwater biodiversity is increasingly challenged by global warming, making it essential to understand the genetic adaptations of keystone freshwater species, such as *Daphnia*, to raising temperatures. Previous studies have uncovered local adaptation of *Daphnia* to higher temperatures. However, the genomic changes underlying this adaptation remain unexplored. Here, we examined genomic differences in *Daphnia cucullata* from five lakes that have been heated by power plants for six decades, resulting in a 2 to 4℃ temperature increase compared to surrounding unheated lakes. *Daphnia* populations inhabiting these heated lakes were compared to populations from seven nearby non-heated lakes. By analysing population structure, introgression patterns, outliers, and adaptive variation rarefaction, we found that *D. cucullata* from heated lakes show significant genomic divergence from conspecifics from control lakes. Outlier SNPs were enriched in temperature-related genes. Although previous studies on the *D. longispina* complex highlighted the importance of hybridization in local adaptation, we found no evidence of introgression in the heated lakes, despite observing first-generation hybrids between *D. cucullata* and *D. galeata*. Our study also indicates that adaptive potential, measured as allelic richness at putatively adaptive loci, was well preserved at the regional scale, while some of non-heated lake populations exhibited low adaptive potential. In summary, our study reveals specific loci involved in thermal adaptation of *D. cucullata*, highlights the independence of evolution of these loci from hybridization, and emphasizes the importance of regional gene pools for local adaptation under global warming.

**Keywords:** *Daphnia cucullata*; landscape genomics; global warming; hybridization; local adaptation

# Post-senescence reproductive rebound in *Daphnia* associated with reversal of age-related transcriptional changes

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A long-lived species of zooplankton microcrustaceans, *Daphnia magna*, sometimes exhibits late-life rebound of reproduction, briefly reversing reproductive senescence. Such events are often interpreted as terminal investment in anticipation of imminent mortality. We demonstrate that such post-senescence reproductive events (PSREs) neither cause nor anticipate elevated mortality. We also found no evidence of a trade-off between clutch size and offspring size in PSRE females, although there was a trade-off between clutch size and neonates' lipid content. We analyze an RNAseq experiment comparing young, old reproductively senescent, and old PSRE *Daphnia* females. We first show that overall age-related transcriptional changes are dominated by the increase transcription of guanidine monophosphate synthases and guanylate cyclases, as well as two groups of presumed transposon-encoded proteins, and by a drop in transcription of protein synthesis-related genes. We then focus on gene families and functional groups in which full or partial reversal of age-related transcriptional changes occur. This analysis reveals reversal, in the PSRE individuals, of age-related up-regulation of apolipoproteins D, lysosomal lipases, and peptidases and of age-related down-regulation of E3 ubiquitin kinases, V-type proton ATPases, and numerous proteins related to mitochondrial and muscle functions. While it is not certain which of these changes enable reproductive rejuvenation, and which are by-products of processes that lead to it, we present some evidence that post-senescence reproductive events are associated with the reversal of age-related protein and lipid aggregates removal, apoptosis, and with restoration of mitochondrial integrity.

**Keywords:** *Daphnia*; senescence; rejuvenation; differential expression; RNAseq

# POSTERS

## Microbiome-mediated adaptation to pollution in urban *Daphnia*

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Urban ponds provide important ecosystem services such as climate regulation, flood control, and promoting biodiversity. However, these ecosystems are particularly sensitive to urban pollution. *Daphnia* are key organisms in pond ecosystems and therefore it is important to study to which extent they are affected by urban stressors, such as pollutants, and if they can locally adapt to these stressors. Their capacity to cope with synthetic chemicals can potentially be influenced by their microbiome and the degree to which the gut microbial communities are able to degrade pollutants. We study to what extent microbiome composition of urban and rural *Daphnia* along well-characterized pollution gradients influences the fitness of *Daphnia* in the presence of pollutants, and to what extent this also depends on *Daphnia* genotype and population origin. We will test *Daphnia* genotypes 15 populations along a pollution gradient. The research includes microbiome transplant experiments followed by tolerance tests and life table experiments in the presence and absence of pollution. The findings will shed light on the mechanisms underlying adaptive trait change dynamics and how these contribute to the overall urban ponds ecosystem resilience.

**Keywords:** urbanisation; pollution; microbiome; adaptation; ponds

# Comprehensive annotation and phylogenetic analysis of Ionotropic Receptors in *Daphnia*

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Chemoreception is the most ancient and widespread sensory modality used by organisms of all levels of complexity. Animals rely on chemical senses to detect food and mating pairs or dangers like predators. Driven by the complexity of chemical signalling cues diverse chemoreceptor gene families evolved encoding proteins which interact with an abundance of chemicals. Among these, ionotropic receptors (IRs) are encoded by a gene family that descents from ligand-gated ion channels. In the freshwater crustacean *Daphnia* these have already been shown to detect chemicals cues released by predators. However, until now most of this diverse gene family and its eco-evolutionary interplay remains largely unknown. To study the molecular basis of *Daphnias'* ability to detect and respond to predators, comprehensive IR gene annotations are pivotal. Already identified IRs (e.g. 85 in *D. pulex*) usually rely on gene models based on automated pipelines without gene model verification. In the current study we identified and annotated IR genes in different *Daphnia* genomes and subsequently performed phylogenetic analyses. Using the *D. pulex* IR set as an initial query for homology-based search approaches, we curated and if necessary, corrected identified genes manually. Verified by the protein domain prediction tools DeepTMHMM and SignalP6, we obtained sets of approximately 70 IR genes per genome which we then analysed phylogenetically. These new sets of IR genes from the curated annotations will now serve as the basis for further evolutionary and functional studies of this chemoreceptor gene family.

**Keywords:** DeepTMHMM; SignalP6; chemoreception; genes; predator detection

# Lipid peroxidation in *Daphnia magna* ovaries by development stage

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Lipids are one of the chief storage nutrients in many organisms. In many animals, including *Daphnia,* lipids containing polyunsaturated fatty acids (PUFAs) are the limiting factor for reproduction, as PUFAs are necessary for proper functioning of cellular membranes and animals usually have limited ability to synthesize PUFAs, relying on their availability in the food. On the other hand, PUFAs are the chief targets for lipid peroxidation (LPO) – an important mechanism of oxidative damage. We investigated the schedule of deposition of LPO targets into developing oocytes by females of *Daphnia magna*, a classic model organism for ecophysiology, by means of fluorescence microscopy in females stained with BODIPY C11 fluorescent dye serving as a specific LPO probe. We observed that, across 3 genotypes tested, the LPO signal was significantly higher in females exposed to the probe during the late stages of ovaries development, relative to earlier stages. There were no significant genotype-by-ovaries-stage interactions. This finding indicates that *Daphnia* females postpone depositing of PUFA-containing lipids during oocyte provisioning until late in the ovary cycle. We hypothesize that such delay is adaptive as it reduces the likelihood of the mutagenic effects of LPO products on the oocyte's genome.

**Keywords:** *Daphnia*; lipid peroxidation; ovaries; BODIPY probe

# Community-wide micro-evolutionary adaptation to anthropogenic stress: context dependencies and ecological implications

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Natural ecosystems are changing rapidly due to anthropogenic influences, causing physiological stress in organisms. Rapid micro-evolutionary adaptation is increasingly recognized as a mechanism that enables organisms to deal with increased stress levels. Most studies in the field of eco-evolutionary dynamics and rapid evolutionary adaptation have focused on single species, while natural communities consist of populations of multiple species that may differ in their adaptive potential, and that potentially affect each other's evolutionary trajectories through ecological interactions. Therefore, in this research we specifically adopt a community perspective by simultaneously addressing the evolutionary response of multiple species. We will test whether the community context affects evolutionary trajectories and vice versa. Aim is to quantify the degree to which rapid evolution mediates the response of freshwater communities (Cladocera) to salinization, a major anthropogenic stressor. By combining an evolution experiment with common gardening experiments in outdoor mesocosms, we will test if evolution contributes to a dampening of stressor effects on individual populations, compositional turnover, biodiversity and ecosystem functions.

**Keywords**: genetic adaptation; eco-evolutionary dynamics; salt stress; cladocera; community ecology

# Microbiota-dependent and independent production of L-Dopa in the gut of *Daphnia magna*

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Host-microbiome interactions are crucial for the functioning of the host organism. In particular, neurotransmitters are commonly implicated in host-microbiome communication, yet the molecular mechanisms of this communication remain largely elusive. Using *Daphnia magna* as a model species, we explored the potential role of the gut microbiome in a peripheral catecholamine pathway, specifically focusing on dopamine production. We found evidence supporting the production of L-Dopa, a neurotransmitter and precursor for dopamine, by gut bacteria. Our findings also demonstrate that both *Daphnia* and its gut microbiome contribute to synthesizing the L-Dopa in the gut. Moreover, there is a peripheral pathway in the gut wall, with a molt-dependent dopamine synthesis, linking the gut microbiome to the daphnid development and growth. These findings suggest a central role of L-Dopa in the bidirectional communication between the animal host and its gut bacteria and translating into the ecologically important host traits suitable for subsequent testing of causality by experimental studies.

**Keywords:** *Daphnia magna*; L-Dopa; Dopamine; gut microbiota

# Distribution, ecology and phenotypic plasticity of *Daphnia inopinata*, the least known member of the *D. similis* complex

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Molecular and morphological studies have revealed that the Old World *Daphnia (Ctenodaphnia) similis* complex comprises several species with various distribution patterns, from regional endemics to widespread taxa, mostly inhabiting temporary pools. The rarest of these, *Daphnia inopinata*, has been described in 2016 from a former military training ground in Germany, based on a few samples collected in early 2000s. Since 2021, we have documented its presence in temporary pools near Prague, and in 2024 at two more sites in Czechia. As is the case for the type locality (where the species is not to be found at present), all new sites are also former military areas (although on the different side of the Iron Curtain), and they are at least partly used as leisure off-road tracks now. We thus assume that the heavy machinery both maintained suitable habitats and contributed to the species' dispersal. In some sampled pools, *D. inopinata* coexists with the notostracan *Triops cancriformis*, which is known to induce defensive phenotypes in other *Daphnia* species. We observed substantial variability in the head shape and development of dorsal spines in both juveniles and adults of *D. inopinata*, apparently corresponding to *Triops* presence. We characterised these traits and compared head shape differences between individuals from natural populations and from clones maintained with or without the presence of *Triops* kairomones in laboratory experiments. While adult specimens with altered head shapes (similar to those also documented from *D. similis*) were only collected from natural ponds, juveniles exhibited pronounced phenotypic changes in both field and experimental samples.

**Keywords:** antipredator phenotypic plasticity; temporary habitats; anthropogenic dispersal; Central Europe; endemic species

# Zooplankton trait distribution patters along urbanization gradients: Berlin as a model system

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Urbanization is currently one of the most pervasive and impactful societal changes of the past decades. Profound environmental changes that result from urbanization lead to dramatic changes in species richness and the species composition or urban compared to rural communities, with potential profound impact on ecosystem functioning. Zooplankton is a pivotal component of pond systems and highly sensitive to environmental changes. In my PhD study, I will test the hypothesis that there is both a general response in community composition to urbanization as well as a local component of urban ecology, where the interaction between urbanization, local environmental conditions and the local food web, determine zooplankton trait distributions. Then, I will quantify to what extent observed community trait distribution patterns can be predicted from the relative abundances of the different species in the community and further explore whether changes in community composition and trait distributions have an important impact on top-down control of algae through grazing experiment. Besides, I will reconstruct how urban communities develop through time, by comparing young versus old urban ponds and by reconstructing changes in community composition from dormant egg banks in sediments. The general aim of my work is to obtain insight on how zooplankton community composition and community trait distributions change with urbanization, and how this impacts ecosystem functioning of urban pond systems.

**Keywords:** urbanization; ponds; zooplankton community; trait distribution; dormant eggs

# Elevated water temperatures increase the fitness of fungal parasite of *Daphnia magna*

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Climate change-induced increases in water temperature pose a threat to the health and resilience of freshwater biota. Organisms living in small water bodies without thermal refugia are particularly vulnerable and might become more susceptible to parasites that thrive in increased temperatures. Previous research on host-parasite interactions has focused on the effect of higher water temperatures on host susceptibility and parasite fitness, but the mechanism leading to such observations remain unclear. Therefore, we conducted a a laboratory experiment using the zooplankton host *Daphnia magna* and its common, virulent fungal parasite *Metschnikowia bicuspidata* in to test if high temperature (a) increases the virulence of parasite transmission stages and (b) affects host filtering rate, and exposure to parasite spores. We pre-incubated parasite spores at 19°C, 23°C, and 27°C for 24 hours and subsequently exposed *D. magna* individuals to these spores at 19°C and 23°C in a full-factorial design. We found that parasite fitness is dependent on both the temperature at which the spores and host are incubated. We observed that transmission stages exposed at higher temperatures exhibited increased fitness parameters, including higher infection rates and spore yield per infected host. This effect remained consistent across both temperatures during which the infection assay was conducted. Host survival in infected individuals is decreased at 23° compared to our 19° treatment. Our research indicates that elevated water temperature enhances the virulence of parasite transmission stages. This work may contribute to our understanding of the mechanism through which temperature influences host-parasite interactions.

**Keywords:** climate change; disease spread; temperature; host-parasite interaction; *Metschnikowia*

# Enhancing *Daphnia*'s tolerance to toxic cyanobacterial blooms: gut microbiome manipulations for increased resilience of freshwater ecosystems

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Our study aims to enhance *Daphnia*'s resistance to toxin-producing cyanobacteria through microbiome manipulation. *Daphnia* adapt to various stressors, including microplastics and pesticides, primarily influenced by their gut microbiome. Recent studies have elucidated the gut microbiome's role in enhancing *Daphnia's* resilience to toxic cyanobacteria, yet the specific microbial contributors remain unidentified. This research aims to pinpoint bacterial strains in *Daphnia*'s microbiome that improve tolerance to toxic *Microcystis* sp. and other microcystin producers, potentially directing microbiome evolution towards more resilient *Daphnia* clones. We propose using probiotic treatments to boost this resilience, promoting the restoration of freshwater ecosystems that are affected by cyanobacterial blooms. Our approach involves a large-scale manipulation of susceptible *Daphnia* sp. microbiomes. This is because we aim to provide a nature-based solution for mitigating harmful cyanobacterial blooms. This research not only addresses gaps in understanding microbiome adaptability under environmental stressors but also explores how these adaptations can improve host fitness and ecosystem recovery.

**Keywords:** Cyanobacteria; *Daphnia*; probiotics; ecotoxicology; microbiome

# First report of *Daphnia* in Hawai'i: An apparently obligately asexual *D. obtusa* population in a fishless temporary reservoir

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A dense population of *Daphnia obtusa* was found on Hawai'i Island in March 2023. No species of *Daphnia* have previously been reported from anywhere in the Hawaiian archipelago in surveys taken between 1886 and 1935 and in 2012. COI and 12S sequencing of individuals preserved in 95% EtOH identify the population as *D. obtusa* group, most closely related to lineages from North America, though still somewhat distinct suggesting possible evolution after arrival in Hawai'i, or at some (as yet unknown) intermediate location. The population, which occurs in a temporary flood retention pond, Paiakuli Reservoir, Waimea, appears to be obligately parthenogenetic, although this reproductive mode has not previously been reported for *D. obtusa*. Individuals were first collected as the reservoir was drying down following a wet period, and were comprised entirely of females (no males found despite careful searching), most of them carrying large clutches of subitaneous eggs, and none carrying ephippia. Four days later the pond had dried completely and the drying mud at what had been the pond bottom was covered with recently dead females, a great many with ephippia each containing two diapausing eggs. Sediment collected and allowed to dry for a month, was then exposed to filtered pond water. Neonate *Daphnia* that hatched after two to four weeks were cultured, becoming large populations the lab. When these became dense, females switched to making ephippia in large numbers without any males being found despite careful searching, leading to the conclusion that the population is obligately asexual.

# The effect of increased DOC concentration on resource use and life history of *Daphnia* – insights from compound-specific stable isotopes

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Many lakes are experiencing browning due to increased loading of dissolved organic carbon (DOC) from the catchment. DOC can provide an additional, allochthonous, food source for lake zooplankton via the microbial loop. We performed a 14-day laboratory experiment on *Daphnia magna* to study the effects of increasing concentrations of DOC (0-90 mg/L) on zooplankton, and also analyzed stable isotopes of carbon ($\delta^{13}C$) in fatty acids (FAs) and amino acids (AAs) to estimate their resource use. *D. magna* was fed the mixotrophic phytoplankton *Cryptomonas* sp., which were grown in corresponding DOC concentrations, and the media was labelled with $NaH^{13}CO_3$ to facilitate the separation of the basal energy sources (DOC and autotrophic production by phytoplankton) in subsequent stable isotope analysis. DOC had a positive effect on *D. magna* reproduction, suggesting that they can utilize this additional resource. This was supported by increasing amounts of bacterial fatty acids (odd-number and iso- and anteiso branched fatty acids) in *D. magna*. According to mixing models employed, over 50% of carbon in *D. magna* FAs and AAs came from the DOC in the highest DOC treatment, and majority of this was via feeding on the mixotrophic phytoplankton. Our study suggests that high allochthony in zooplankton may be attained by feeding on mixotrophic algae without compromising growth or reproduction.

**Keywords:** *Daphnia magna*; browning; *Cryptomonas*; microbial loop

# Predator induced cellular plasticity in *Daphnia longicephala* sensory antennules

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The freshwater crustacean *Daphnia* is well known to adapt to an increased predation risk through the expression of predator specific inducible defenses. The expression of these inducible defenses is initiated upon the perception of predator specific chemical cues, so called kairomones. The aesthetascs located on the antennules serve as a central element of kairomone perception. However, the distinct cellular and molecular mechanism that enable predator perception are not understood. In order to obtain a deeper insight into the involved cells and chemoreceptors, we first quantified the number of olfactory sensory neurons (OSNs) in the antennules in response to predator exposure via immunohistochemistry. In a second step we stained IR25a, a ionotropic coreceptor previously identified to be essential for predator perception. We observed an ~15% increase of OSN number during predator exposure independent of normal cell division in the antennules. In addition, IR25a is more abundant in predator exposed specimen. Our results show cellular plasticity at the OSNs in form of nerve cell number and IR25a abundancy upon predator perception and provide important insights into how the nervous system reacts to the presence of their predators.

**Keywords:** olfactory sensory neurons, chemoreception, phenotypic plasticity

# *Daphnia* is a sentinel species for chemical pollution control in protecting freshwater ecosystems

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*Daphnia* has traditionally been used as a sentinel species for freshwater quality and for setting regulatory limits on chemical pollutants. Yet despite having early access to genome data and resources, there has yet to be much change in technologically transforming hazard detection for better protecting aquatic environments. Here, we apply 'omics' technologies to *Daphnia* exposed to real-world environmental pollutants to identify the biomolecular signatures that are indicative of chemical components in the water that may be harmful to animals, including humans. This added functional genomics knowledge for detecting biomolecular toxicity pathways broadens the application of *Daphnia* as a sentinel for both human health and the environment (One Health Toxicology) under the regulatory banner of Next Generation Risk Assessment (NGRA). This approach aims to identify molecular biomarkers associated with chemical exposure and ecological endpoints, which are evolutionarily conserved among species, providing early warnings of hazardous chemicals, even at environmentally relevant concentrations and mixtures. Here, we propose a framework entitled “Precision Environmental Health” that utilises non-targeted analysis to characterise real-world chemical mixtures and high throughput 'omics' technologies (e.g., transcriptomics and metabolomics) to identify co-response modules activated by these mixtures. This framework employs orthologs within conserved pathways to enable cross-species extrapolation for early diagnosis of chemical pollution hazards, even at sublethal concentrations. In this context, *Daphnia* functions as a sentinel species to identify (i) putative molecular key events predictive of adverse outcomes, (ii) biomolecular signatures of chemical mixtures, and (iii) evolutionarily conserved biomolecular targets of chemical exposure.

**Keywords:** *Daphnia*; water pollution; omics; NGRA; One Health Toxicology

# *Daphnia pulex* as top-down control of phytoplankton in three subtropical shallow lakes

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The study examined the ability of *Daphnia pulex* to regulate the growth of phytoplankton in three small, shallow, and neighboring bodies of water situated in an urban reserve named Pedregal de San Angel Ecological Reserve in Mexico City. Three mesocosms (80 l) were placed in each lake: one control (without *Daphnia*) and two with 80 ind $l^{-1}$ of *D. pulex.* The experiment was conducted during two climatic seasons: cold-dry and warm-rainy. Based on the Carlson and Simpson trophic index (Total phosphorus, chlorophyll concentrations, and Secchi disk depth), the water bodies were classified as meso-eutrophic (North Lake), eutrophic-hypertrophic (Central Lake), and hypertrophic (South Lake). *D. pulex* maximum density values reach around 300 ind $l^{-1}$ at the end of the cold-dry season experiment. In all cases, the presence of *D. pulex* led to decreased chlorophyll *α* concentration values, with the highest average effectiveness observed in the Central Lake (85.5%), followed by the North Lake (74%), and the South Lake (71%). The prevalence of cyanobacteria (*Microcystis* spp.) negatively affected *Daphnia*'s growth and effectiveness in consuming phytoplankton, primarily in the South Lake. The phytoplankton control by *Daphnia* was better during the cold-dry season. In the warm-rainy season, the survival time of *D. pulex* in the South Lake mesocosms was shorter. The study suggests that *D. pulex* could be a viable option for controlling phytoplankton in these ponds, provided its predation by the local fish (Goodeid and carp juveniles) is prevented.

**Keywords:** eutrophication; urban lakes; biomanipulation; mesocosms

# Impact of river valleys on genetic diversity and species distribution patterns of the family Cyprididae (Crustacea: Ostracoda) inhabiting the Tibetan Plateau

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Understanding genetic diversity in aquatic organisms is crucial for assessing ecosystem health and evolutionary processes. Here, we investigated the genetic diversity of Cyprididae populations across 18 waterbodies on the Tibetan Plateau. We sequenced the COI locus in 106 individuals, identifying 25 unique haplotypes with distinct regional distributions. Phylogenetic analysis revealed nine clades, including two cryptic lineages, exhibiting clear geographical patterns. Additionally, mtDNA haplotype networks and nuclear loci phylogenetic analysis supported these findings. Molecular variance analysis indicated significant genetic differentiation among populations within regions. Although pairwise $F_{st}$ values did not correlate with geographical distance overall, significant correlations were found within specific regions. Species composition varied significantly among river valleys, with endemic species contributing to overall diversity. Our study enhances understanding of Cyprididae genetic diversity and highlights the importance of region-specific conservation efforts in maintaining biodiversity on the Tibetan Plateau.

**Keywords:** Genetic diversity, Cyprididae, Species composition, Biodiversity, Tibetan Plateau

# Zooplankton communities under global change – taxon composition and hybridization dynamics in time and space

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Global change influences ecosystems in many facets. Monitoring the drivers and understanding the consequences of climate change is essential to develop management plans and inform stakeholders. Lake Constance, Europe's third-largest lake by surface area, situated on the borders between Switzerland, Germany, and Austria. Over the past century, the lake experienced significant eutrophication due to wastewater disposal and phosphorus-containing detergents, which was reversed following the installation of water purification systems and a ban on such detergents. These nutrient fluctuations impacted the lake's species composition, particularly the plankton communities. Among the zooplankton, *Daphnia* species are crucial to the lake's food web. During eutrophication, *Daphnia galeata* invaded the lake, hybridizing with and outcompeting the native *Daphnia longispina*. After re-oligotrophication, *D. galeata* nearly vanished but persists in resting egg banks. Recently, *Daphnia cucullata* invaded, displacing the reestablished *D. longispina* and hybrid populations during summer. The mechanisms behind this displacement remain unclear but coincide with the invasion of three-spined sticklebacks into the pelagic zone, quagga mussels colonizing the lakebed, and lake warming. The ecological consequences of these changes on Lake Constance's food web are not fully understood. This project aims to: 1) investigate the spatio-temporal dynamics of *Daphnia* communities in Lake Constance and assess the conditions favoring each species' dominance, 2) identify potential hybridization events among *Daphnia* species, and 3) evaluate the impacts of hybridization and the invasions of sticklebacks and quagga mussels on the food web. These objectives will be pursued through bi-weekly sampling campaigns, whole-genome sequencing, and laboratory and mesocosm experiments.

**Keywords:** *Daphnia*; Climate change; eutrophication; hybridization; spatio-temporal dynamics

# Utilizing genomic and phenotypic data with machine learning to uncover evolutionary insights in *Daphnia* population

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The genetic architecture of adaptive traits is crucial for predicting evolutionary responses. Most adaptive traits are polygenic, and examining how these genetic changes enable organisms to adapt to their environments, including responses to climate change and other environmental pressures, is essential for biodiversity conservation and ecosystem management. In that sense, understanding the genetic architecture of temperature adaptation is key for characterizing and predicting the effect of climate change on natural populations. To explore these dynamics, this study leverages both genomic time-series to investigate the genetic mechanisms underlying environmental adaptability and resilience in aquatic ecosystems and phenotypic data from *Daphnia* populations to provide real-time, high-precision detection and classification of *Daphnia*, offering insights into their adaptive phenotypic strategies. Machine and deep learning algorithms offer a new avenue for analyzing such data by employing advanced computational techniques to sift through complex datasets, identifying patterns and correlations that might not be immediately apparent to human researchers. This study specifically explores the application of Neural Networks (NNs) to analyze the data, focusing on developing methods that enhance efficiency and robustness while improving the interpretability of the techniques used, providing insights into evolutionary strategies in the face of a rapidly changing environment.

**Keywords:** genomics; *Daphnia*; machine learning; environmental adaptability; evolutionary biology

# Evolutionary ecology of pollutant tolerance in urban *Daphnia*

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Urban areas are expected to grow in the future and are sources of increased stress and health risks for ecosystems and people alike. The functionality of urban pond ecosystems is threatened by the potential influx of numerous pollutants from different sources, e.g. insufficiently treated wastewater and urban runoff. Especially synthetic organic chemicals, such as pesticides and vehicle-related compounds pose a threat to urban freshwater systems, as they can persist in the water cycle and affect aquatic organisms. *Daphnia magna* has recently become a promising model system for urban evolution studies. Their fitness, grazing abilities and species composition have been shown to be influenced by urban stressors, affecting the top-down control of (toxic) phytoplankton blooms. Yet the effect of urbanisation-associated pollution on *Daphnia magna* populations is mostly unknown. We plan to identify mechanisms of how urbanisation-related pollution affects the evolutionary adaptation of *Daphnia magna* populations to urbanisation. First, we will identify dominant pollutants in focal urban and rural ponds and conduct an experiment to quantify genetic variation in tolerance to urbanisation-associated pollution. Using a microcosm study combined with an integral projection modelling approach, we will quantify sublethal effects on selected lineages of target urban pollutants. Lastly, we will perform an in situ reciprocal transplant experiment to identify to what extent *Daphnia* populations are adapted to the specific pollution composition of their pond of origin. Our study will aid in determining the threat of urban pollutants to pond ecosystems and quantify to what extent *Daphnia magna* populations genetically adapt to this threat.

**Keywords:** Urbanization; IPM; Life History Traits; Ecotoxicology; Water flea

# Effects of the submerged macrophyte *Stuckenia pectinata* on life history and feeding of *Daphnia* and *Simocephalus*

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Macrophytes and cladocerans are the main antagonistic groups that control phytoplankton biomass; however, the mechanism behind this interaction is not completely clear. In laboratory settings, the effect of the submerged macrophyte *Stuckenia pectinata*, its exudates, and associated microbiota + exudates on the life history of cladocerans until their first reproduction was determined. Additionally, filtration and consumption rates of cladocerans exposed to *Stuckenia* exudates were separately determined using seston from a lake in central Mexico. Bioassays revealed that the age at first reproduction (9.4 days) and second molt (3.5 days) of *Simocephalus* are faster in the presence of plant exudates. However, it produces fewer offspring (4.8 organisms) compared to *Daphnia* (8, 3.1 days, and 6.7 organisms). Furthermore, feeding rates showed that only *Daphnia* exhibited a difference, consuming a higher amount of algae (between 40 to 60%) from the genera *Desmodesmus* (both small and large size), *Cyclotella*, and *Coscinodiscus* in the presence of *Stuckenia* exudates, while there was no difference in phytoplankton consumption for *Simocephalus*. Exposure to *Stuckenia* exudates resulted in earlier reproduction, fewer smaller neonates, and slower growth in *Daphnia*, implying a trade-off between these variables and their high filtration and consumption rates; however, further investigation of assimilation rates is important.

**Keywords**: Cladocera, Biomanipulation, Consumption Rate, Filtration Rate, Life Tables

# Do *Daphnia* males really live shorter than females?

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The observation made nearly a hundred years ago that *Daphnia* males, in similar conditions, live significantly shorter than females was one of the foundations of the rate of life theory of longevity and aging. However, more recent data, particularly in conjunction with the effects of caloric restriction on longevity, cast doubts on the universality of this observation. Specifically, it is possible that, when kept at the same food level and density as females, *Daphnia* males, being smaller, experience ad libitum food availability and therefore exhibit shorter lifespan than food limited females. We measured lifespan of males and females of two clones of *Daphnia magna* over a range of food levels spanning from near-starvation to ad libitum and detected sex-by-food interactions over the caloric restriction range in both clones and over the starvation range in one of the two clones. However, while the two sexes responded to food levels differently, males showed a shorter lifespan consistently across all food levels, contradicting the hypothesis of caloric restriction mediated longevity differences. These data provide the baseline for caloric restriction-mimicking interventions aiming to extend lifespan in this model organism.

**Keywords:** caloric restriction, longevity, gerontology, aging, sex differences

# Taxonomical analysis of *Simocephalus* from central Mexico: the importance of morphometric, molecular, and ecological approaches

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The genus *Simocephalus* is essential to the freshwater ecosystems' littoral zone. Nonetheless, its taxonomic determination is quite confusing due to the gap in knowledge in America and its complex life cycle morphology. We analyzed the diversity of *Simocephalus* species in Central Mexico's lakes, reservoirs, and wetlands using morphological and molecular (COI gene) characteristics. We also determined the limnological and biophysical properties of the sampled waterbodies. Morphologically, we found one population of *S. exspinosus*, three populations of *S. serrulatus,* and seven populations of *S. mixtus* with at least two well-defined groups (four populations of *S. mixtus* and three of *S.* cf. *mixtus*). The differences between these groups are body size, ocellus shape, and frontal part of the head. COI genotyping corroborated these distinctions, revealing at least 10% group dissimilarity. Environmental ordination by principal components analysis explained 85.9% of the variation, with chlorophyll *a*, and conductivity explaining most of the variability. *S. serrulatus* preferred high-altitude and low-conductivity environments, while *S. mixtus* did not exhibit any habitat specialization. In laboratory, we monitored ontogenic morphological changes using 13 morphometric characters over 80 days in four populations from two morphospecies of *S. mixtus* group, including a Peruvian strain. Discriminant analysis separated two groups of *S. mixtus,* coinciding with morphological and molecular data, revealing that the most significant morphometric measures in the ordination were the upper margin of the eye to the most post-dorsal length and the base of prominence. Our findings enhance the taxonomy and ecological insight of *Simocephalus* in Central Mexico, emphasizing integrated approaches.

**Keywords**: Anomopoda, COI gene, Environment, Morphometry, Integrative Taxonomy.

# Thermal stratification and phenology of Cladocera in Lake Maggiore: comparison between two decadal series

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Lake Maggiore during the last 10 years experienced a sharp increase in water temperature and a more pronounced thermal stratification, making it an ideal site for investigating the impact of global warming. A monitoring program, including the entire vertical profile of the water layer in which zooplankton is mostly distributed along with detailed analysis of the plankton community, allowed the detection of a multi-decadal trend in changes in phenology and abundance of the different zooplankton taxa. In this contribution we focused on cladocera, reporting the changes that occurred during the last 10 years, when water temperature of the euphotic layer increased by ca. 2.3 °C. The observed changes are consistent with a general trend towards smaller zooplankton taxa, likely better adapted to grow at higher temperatures and in more superficial water layers. Overall, these changes tend to mimic patterns observed during lake eutrophication, proving an overlapping of responses between climate driven and trophic conditions manifest in the zooplankton community.

**Keywords:** global warming**;** Lake Maggiore; phenology; thermal stratification

# Differential responses of *Ceriodaphnia cornuta* to different chemicals in presence of microplastics

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In aquatic ecosystems, Microplastic (MP) has emerged as an ubiquitous contaminant affecting different cladoceran species by directly impacting ecophysiological attributes and also acting as a vector for dissolved chemicals, being sorbed onto the hydrophobic MP surface. This paper explores the chronic responses of tropical Cladocera, *Ceriodaphnia cornuta* to MP (size 11-20µm), important plastic additive bisphenol-A, widely used agrochemical mancozeb and MP preconditioned with either bisphenol-A or mancozeb. The chronic tests were conducted with environmentally relevant concentrations of mancozeb (1µg/L), bisphenol-A (1µg/L) and MP (11-20µm; 500 particles/L). The LC25 value in the standard acute toxicity assay was 386µg/L and 163 µg/L, for bisphenol-A, and mancozeb, respectively. Chronic exposure revealed that both survival and reproduction were differentially affected by presence of MP, MP in combination with the chemicals, and MP preconditioned with chemicals. MPs preconditioned with mancozeb incurred 100% mortality in *C. cornuta*, within 11 days of initiation of experiment, whereas the cohorts recorded longer survival in either MP or mancozeb medium. Reproduction was significantly higher in bisphenol-A , among all treatments, but lower than the control. Reproduction in bisphenol-A preconditioned MP, was the highest among all treatments with MP in the medium. The life expectancy at birth, was higher in bisphenol-A preconditioned MP, among all treatments. Age at first reproduction was significantly delayed in the treatment medium combining bisphenol-A, mancozeb and MP. These results indicate that MPs present in the aquatic environment, may incur differential effects depending upon the other contaminants in the water.

**Keywords:** microplastic; Mancozeb; Bisphenol A; *Ceriodaphnia cornuta*

# Impact of warming on parasite diversity: comparative metagenomics of *Daphnia cucullata*

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Freshwater temperatures have risen by 0.34°C per decade in the last 100 years, with significant impacts on infectious disease ecology and ecosystem health. Understanding the complex effects of warming on parasite ecology and evolution requires investigation in ecologically relevant, experimental settings. We are studying a system of natural lakes near Konin, Poland that have been artificially heated by an average of 2-4°C after receiving hot water discharge from nearby power plants for the last 60 years. We will test the hypotheses that warming (a) reduces diversity of parasites in *Daphnia cucullata*, a keystone zooplankton species, and (b) exerts positive selection pressure on the parasites' loci that may confer thermal adaptation, e.g. on genes coding for heat shock proteins. To date, we have performed shotgun DNA-sequencing of six to eight individual *D. cucullata* each, sampled from five heated and seven control (i.e. nearby, non-heated) lakes, resulting in 92 metagenomes. Preliminary analyses suggest the presence of four obligate parasites of *Daphnia*, representing broad range of taxa: *Caullerya mesnili* (Class: Ichthyosporea), *Mitosporidium daphniae* (Phylum: Microsporidia), *Daphnia Iridescent Virus* (DIV-1, Family: Iridoviridae; causative agent of the virulent White Fat Cell Disease), and *Pasteuria ramosa* (Phylum: Bacillota). Our investigation would contribute to a fundamental understanding of the effects of warming on parasite ecology and evolution.

**Keywords:** host-parasite interaction; metagenomes; global warming; genetic diversity; climate change

# Taxonomic change and hybrid genome evolution in the *Daphnia longispina* complex

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Secondary contact and hybridization of diverged lineages can lead to various evolutionary outcomes, especially during periods of ecological disruption. The *Daphnia longispina* complex, occupying different ecological niches in peri-Alpine lakes, have faced substantial changes due to historical eutrophication and recent climate shifts and serves as an ideal model to study the patterns and evolutionary consequences of hybridization. This project aims to investigate the spatial and ecological factors influencing the distribution and genome evolution of the *Daphnia longispina* complex and their hybrids in peri-Alpine lakes. I aim to assess whether hybridization has left a permanent mark in the genomes of the species after several decades of ecological changes and to identify mechanisms driving hybrid genome evolution. For this, I collected *Daphnia* samples along with measurements of abiotic and biotic factors from 43 peri-Alpine lakes. Together with historic data, I am analyzing ecological data to assess historic and current environmental variation of these lakes. From the collected *Daphnia* samples, I am generating whole-genome re-sequencing data for 20 individuals per lake. From these data, I will identify the current taxonomic composition and genomic regions affected by hybridization to better understand drivers and repeatability of hybrid genome evolution. The project will shed light on environmental and spatial factors shaping hybridization and genome evolution, setting the stage for investigating the eco-evolutionary consequences of hybridization. Integrating genomic data with environmental and historical variables, I aim to contribute to our understanding of the dynamics of ecological change and hybridization and inform conservation strategies for aquatic ecosystems.

**Keywords:** hybridization; eco-evolutionary dynamics; *Daphnia*

# Dearbhla, the *Daphnia*, and her research highlights

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*Daphnia* are a fantastic model organism for research, but also for research communication. Dearbhla, the *Daphnia*, is a human-sized *Daphnia* in the form of a costume designed to engage the public in environmental research. Dearbhla is a ‘spokes-species’ for environmental pollution, and the research that is currently underway to explore and remediate environmental issues. Dearbhla is a custom mascot design, encompassing the key features of a *Daphnia* including swimming appendages, singular eye and brood pouch and her unique appearance attracts a lot of attention. Through this project, we can bring *Daphnia*, quite literally, into the public domain at Science Festivals, open days, art exhibitions and other public events to spark discussion and support other hands on activities to create a memorable and engaging day. Some research highlights shared through Dearbhla include the ‘*Daphnia* Detectives’ project, where some pillowcases, pegs and pink particles showcase how every day items can be combined with research materials to create an interactive public engagement or schools outreach activity. The project consists of a bank of open access online resources that cover the topic of microplastics and *Daphnia,* from experimental design stages through to example results and data sets that can be used to explore data analysis themes within the high school curriculum context. Through this project we can spark discussions on the importance of water quality, moving beyond an anthropocentric view to explore the ecosystem level consequences of this in an engaging and novel way, aligning with the increasing public interest in recent years.

**Keywords:** environmental science; science communication; public engagement; outreach; scicomm

# Does carapace thickness provide any defense for cladocerans against *Hydra vulgaris* predation?

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*Hydra*, a predatory macroinvertebrate, exerts a significant influence on the composition and abundance of cladoceran populations. These microcrustaceans have evolved a variety of strategies to evade predation, including behavioral, demographic, and morphological adaptations. Extensive research has focused on the induction of defensive structures like helmets and spines in *Daphnia*, which lead to carapace thickening. However, the role of carapace thickness in influencing *Hydra*'s prey selection among cladocerans remains poorly explored. This investigation assessed the impact of carapace thickness on predator-prey dynamics involving seven cladoceran species (*Ceriodaphnia dubia, Daphnia mendotae, Simocephalus mixtus, Alona guttata, Scapholebris freyi, Pleuroxus varidentatus,* and *Macrotrix triserialis*) and three populations of *Hydra vulgaris*. Carapace thickness was evaluated using histological techniques, while the impact of *Hydra*'s cnidocytes was examined through scanning electron microscopy. *Alona guttata*, with the thickest carapace (8.93 ± 0.69 µm), was most frequently preyed upon, whereas *Ceriodaphnia* and *Simocephalus* were least consumed. The study further investigated *Hydra*'s feeding behavior on *Alona* and *Simocephalus*, focusing on the predator's attachment orientation (vertical or horizontal). Feeding behavior tests indicated that horizontally attached *Hydra vulgaris* encountered *Simocephalus* more frequently than *Alona*. Nevertheless, fewer than half of these encounters led to ingestion, and some *Alona* individuals died without being consumed. In contrast, vertical attachment resulted in more frequent and successful predation on *Alona*. Furthermore, differential counts of cnidocytes were observed when adhering to or penetrating the carapaces of cladocerans, and penetration by tubules was also documented. The findings suggest that carapace thickness does not determine prey selection by *Hydra vulgaris*.

**Keywords**: Histology, Feeding behavior, Food preference, Scanning Electron Microscopy.

# Diversity, distribution, and abundance of cladocerans from the eutrophicated dam of Valle de Bravo, México: An annual study

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The Valle de Bravo dam is part of the Cutzamala system, a series of dams that supply drinking water to some municipalities in the Metropolitan Area in Mexico City Valley. This monomictic reservoir displays accelerated eutrophication due to the constant supply of nutrients from agricultural areas and aquaculture farms from the tributaries rivers. This supply of limiting mineral nutrients has provoked a condition of permanent Harmful Cyanobacterial Blooms (HCBs) with the consequent deterioration of water quality. This study aimed to determine cladocerans' distribution, abundance, and diversity during an annual cycle. The samples were obtained monthly in six sampling sites. In total, 12 cladoceran species were identified, and eight species were new records for the Valle de Bravo reservoir. The highest density of cladocerans was observed in March and June, with a remarkable decrease from August to November. The sampling sites S1, S2, and S4 showed cladocerans densities higher than 60 ind. $L^{-1}$. *Bosmina longirostris* had the highest density in S1, and six species were collected all year, despite the cladoceran density we observed being lower than that registered in other studies. The reservoir of Valle de Bravo is eutrophicated, and the cyanobacteria are permanently blooming, affecting the survival of cladocerans; nevertheless, the species richness was higher compared with other waterbodies in Mexico. The changes in cladocerans' density and the presence or absence of some species could warn about the deterioration progress of this and other eutrophicated reservoirs.

**Keywords:** eutrophication, zooplankton, diversity index, tropical lake, HAB

# Late maturation in the eye pigmentation of *Moina cf. macrocopa*: neonates with red color eye

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Eye pigmentation in cladocerans is accomplished during embryo development, and when the neonates are released for the incubation chamber, they have the typical black eye color. There is no information that the full pigmentation of the eye is accomplished after birth. In this study, we observed the changes in the pigmentation of the eye of *Moina* cf. *macrocopa* related to different culture volumes and food concentrations. The Chlorophycean algae *R. subcapitata* was used as food. When *Moina* was reared in 30 mL, the offspring with a red eye was 97.5%. In assays performed in 2 mL culture volume in 24-well microplates with different food concentrations, when feeding with 12, 6, and 3 X$10^6$ cells mL$^{-1}$, the neonates with the red eye were 96.6, 56.4, and 53.3%, respectively. After 72 hours, all the neonates born with red eyes, the eye pigmentation changed gradually to black. The pigmentation of the eye in the progeny of this “atypical” *Moina* had different tones, ranging from pale red to bright red. Nevertheless, it is remarkable that all neonates hatched from the ephippia of this cladoceran had the eye black. In the newborns of the strain of *Moina* we studied, the red eye was more common than the black eye and was related to some rearing conditions. It is necessary to determine if the reddish color in newborns could modify elemental behavioral responses, such as light's attraction/avoidance effect or the fitness of neonates born with this trait.

**Keywords:** Moinidae; Cladocera; eye development; reproduction; embryonic development

# *Daphnia obtusa* affects environmental DNA stability

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Environmental DNA (eDNA), nowadays, has become an extensively used tool for both the rapid detection and the monitoring of a wide range of organisms (such as invasive allochthonous species) in freshwater ecosystems. The applicability of this technique depends on different abiotic and biotic factors that influence the integrity and availability of eDNA. Zooplankton releases a variety of enzymes, including DNases, into the surrounding water, thus, possibly affecting eDNA stability. This study evaluates the impact of zooplankton on eDNA degradation, using *Daphnia obtusa* as a model organism and the DNA obtained from three invasive species found in Lake Maggiore, *i.e.*, the bryozoa *Pectinatella magnifica*, the crayfish *Procambarus clarkii,* and the fish *Silurus glanis*, as eDNA source. The presence of *D. obtusa* resulted in eDNA degradation. These results suggest that zooplankton can reduce the quantity of eDNA in freshwater and hamper its detection, especially when in high abundance.

**Keywords:** zooplankton; eDNA; invasive species; Cladocera

# Wetlands as harbors of regional species diversity in Cladocera

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Natural wetlands in Central Europe decreased in size over the course of the 20th century, partially due to destruction for agriculture and partially due to deterioration from human activities such as drainage and eutrophication. The dominant type of lentic environment is now represented by fish ponds, which have been built by humans since the 11th century. While their primary functions are fish production and water retention, non-intensively managed fish ponds can substitute for natural wetlands in supporting regional biodiversity, especially when they have well-developed littoral zones. However, the management of fish ponds varies greatly from year to year based on the size and number of fish, resulting in conditions that can range from excellent to very poor in the same pond with respect to biodiversity. Additionally, there is little care for the littoral zones, and many ponds have lost their littoral/wetland areas over the years, often becoming overgrown with willows. In our study, we selected 18 fish ponds in Třeboňsko PLA with varying degrees of overgrowth and 27 sites representing three types of the most common wetland zones: (1) low wetland vegetation, (2) reed beds, and (3) willow growths. The project focuses on the biodiversity of various taxonomic groups, including birds, selected insect orders, spiders, zooplankton, and selected groups of plants. In this presentation, we discuss Cladoceran communities and the importance of flooded littoral zones for their regional species diversity.

**Keywords:** wetlands; zooplankton; biodiversity; littoral; fish ponds

# Assessing the effects of medicinal cyanobacterial diets on different strains of a fungal parasite of *Daphnia*

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Parasite epidemics can influence water quality, nutrient flow and aquatic community composition. Gaining insights into how biotic and abiotic interactions impact both genetic and phenotypic diversity of parasites is important for understanding disease dynamics in nature. In the *Daphnia-Metschnikowia* host-parasite system, we are beginning to understand what drives susceptibility of hosts to this parasite and how cyanobacterial diets modulate this susceptibility. Here, we asked if medicinal cyanobacterial diets have the same impact on different strains of *Metschnikowia*. This fungal parasite is known to have little genetic diversity in the wild. Thus, it is not surprising that this parasite also shows small phenotypical differences such as in infectivity, as multiple studies have reported that susceptible hosts can be infected by different strains of this parasite. We designed two infection assays with different goals. First, we exposed four different clones of *Daphnia magna* to three different strains of *Metschnikowia bicuspidata,* fed *Daphnia* high-quality green algae and followed their infections. This assay aimed to quantify parasites trait variation under a high-quality diet. Preliminary data suggest that parasite strains differ in traits under high quality food conditions. Notably, the strain with the lowest infectivity also produced larger spores compared to the other two strains. We will address how parasite and hosts traits change under cyanobacterial diets on a later experiment. Investigating how parasite traits are influenced by cyanobacterial diets and the mechanisms driving such variations will increase our knowledge of the factors that drive epidemics in the wild for this parasite.

**Keywords:** host-parasite interactions; *Microcystis*; *Metschnikowia*; parasite traits; hosts resistance

# Microbiome-mediated metal toxicity in *Daphnia magna*: A focus on hexavalent chromium

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A healthy microbiome is essential for maintaining the overall well-being of the host. However, exposure to xenobiotics can disrupt microbiome composition and diversity that could lead to adverse effect on host physiology, impacting metabolism, immunity, development, and behavior, and ultimately reducing the host's ability to tolerate toxic insults. This study investigates the role of the microbiome in influencing metal toxicity in *Daphnia magna*, with a focus on hexavalent chromium ($Cr^{VI}$). An acute OECD assay was conducted on two clones of daphnids, comparing individuals with an intact microbiome to germ-free counterparts. Differences in $Cr^{VI}$ body burden and toxicity tolerance were analyzed to explore the relationship between the microbiome and metal toxicity. The 48-hour LC50 analysis also revealed that germ-free daphnids were significantly more resistant to $Cr^{VI}$ toxicity. We also observed that microbiome facilitates $Cr^{VI}$ accumulation, with lower $Cr^{VI}$ levels observed in the germ-free daphnids. These findings suggest that the microbiome affects both Cr accumulation and its toxicity in *Daphnia magna*, contributing to disease pathogenesis. Therefore, microbiome variability can contribute to the outcome of the toxicity assays.

**Keywords:** *Daphnia magna*, microbiome; hexavalent chromium; toxicology

# Combined Effects of Temperature and Chemical Exposure: Insights from *Daphnia magna* using a degree-days approach

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In aquatic environments, organisms face a multitude of stressors, including thermal and chemical exposures. As water temperatures are rising due to climate change, the thermal pressure on biota intensifies. Because temperature influences virtually all physiological functions and biological response depends on exposure duration, the test temperature may alter both the duration as physiological time and chemical toxicity via toxicokinetics. Here, we used a novel combination of the degree-days (DD, proxy for physiological age) approach and the chemical activity concept to evaluate the interactions between chemical exposure and thermal stress in *Daphnia magna*. Daphnids were maintained at two temperatures (20˚C and 25˚C) and exposed to chemicals inducing narcosis (activity range 0.01 – 0.1; polycyclic aromatic hydrocarbon mixture). We hypothesised that (H1) temperature would not affect outcomes when the exposure time is defined in degree-days (60 DD), and (H2) higher lethality (assessed as median lethal activity $La_{50}$) would occur at the higher temperature when the exposure time is defined in calendar time (3 days). Both hypotheses were confirmed as we found no significant temperature effect when the exposure time was adjusted to DD ($La_{50}$: 0.061 vs 0.068) and significantly higher toxicity at 25˚C compared to 20˚C ($La_{50}$: 0.035 vs 0.068) after 3 days of exposure in both treatments. These findings suggest that the temperature effect was exclusively attributable to differences in exposure duration, with no significant temperature x dose interaction. Thus, to understand the mechanisms of thermal stress in conjunction with other factors, the physiological age of the experimental animals should be considered.

**Keywords:** thermal stress; degree-days; exposure duration; physiological age; acute toxicity

# *Daphnia magna* and microplastics: a multi-generational study assessing microbiome effects and microbial degradation

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Plastics, inexpensive and moldable, have revolutionized many sectors ranging from healthcare and industry to agriculture and households. In 2016, over 10% of global plastic waste entered waterways, with projections suggesting that aquatic environments could contain more than 80 metric tons of plastic by 2030. Larger plastics break down into smaller fragments, with particles under 5mm classified as microplastics. Studies revealed that freshwater reservoirs, particularly near urban areas with high human activities, accumulate high concentrations of microplastics. This prevalence raises important questions about how bacteria interact with these plastics - potentially using them as carbon sources or substrates that facilitate degradation and integration into aquatic food webs and elemental cycles. Building on this, various aquatic organisms at different trophic levels, ingest microplastics, potentially affecting the bacterial community residing on and within them (i.e. the microbiome). Filter feeding organisms – such as *Daphnia magna* – are particularly susceptible to microplastic ingestion, as microplastics are often similar in size and shape to their natural diet. Therefore, *Daphnia* is an interesting model to study interactions between microplastics and the microbiome and identify risk and biodegradability of microplastics. In this study, we exposed *Daphnia* to both bioplastics (PLA – polylactic acid) and traditional plastics (PET – polyethylene terephthalate) over the course of one year and spanning multiple generations. This experiment allowed us to study changes in the microbiome community, identify key gene functions involved in plastic degradation pathways through whole metagenome sequencing, and isolate specific bacterial strains involved in degradation via in vitro biodegradation assays.

**Keywords:** *Daphnia magna*; microbiome; microplastics; whole metagenome sequencing; plastic degradation pathways

# The only terrestrial waterflea genus and its unusual adaptations to living in rainforests: new discoveries in *Bryospilus* Frey, 1980 (Cladocera: Chydoridae)

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Cladocera are generally found in "conventional" waterbodies, yet there are some that actually climb trees. *Bryospilus* Frey, 1980 (Cladocera: Chydoridae) is unique within the class Branchiopoda. It is the only waterflea lineage of genus level that is specialised to living in wet moss and phytotelms, disconnected from larger waterbodies. To survive in this ("semi-subterranean") extreme environment where rarely other aquatic microcrustaceans (besides harpacticoids) can be found, the species of this genus have a special phenotype, characterized especially by reductions. We studied the morphology and live behaviour of a new species of *Bryospilus* found in moss habitats in India (Bharat), the first ever found in the Oriental Region. The new species is remarkable within the genus, with less reductions, which lead us to allocate it to a new subgenus. The new species provides important clues on where *Bryospilus* is situated in the cladoceran Tree of Life, but also it helps us to understand the evolutionary steps necessary for microcrustaceans to explore unusual "terrestrial" microniches such as wet moss patches in rainforests.

**Keywords:** evolution, behaviour, taxonomy, *Bryospilus*, terrestrial

# (Meta)barcoding the Baltic Sea Cladocera using MinION technology: diet, diversity and origins

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Cladocera of the Baltic Sea occupy key roles within this economically important ecosystem. Since recent decades, several invasive cladocerans have become part of the pelagic and littoral areas of the Baltic Sea. Although this large waterbody is well studied, our knowledge about the current diversity, origins and diet preferences of the residing Cladocera, still has major gaps. We examined the Cladocera of the Gulf of Finland in detail, assessing their morphological and molecular diversity in a first step (using COI and ITS genes). Creating a new database of reliable cladoceran barcodes for the Gulf of Finland (and surrounding waters), has allowed us to compare patterns of origins of the Baltic Sea Cladocera, indicating a complex colonisation history for several species (including multiple genotypes and cryptic species) and some general trends. Building on our new barcode database, we applied MinION$^{TM}$ technology for metabarcoding, assessing the overlap in diets of the invasive and non-invasive predatory Cladocera of the Baltic Sea. Our approach, which has never been used before to examine zooplankton diets, leads to interesting results and some hitherto unknown potential ecological impacts of the cladocerans in the Baltic Sea ecosystem. We discuss the benefits and pitfalls of the approach, which can be applied to investigate cladoceran roles in any aquatic ecosystem. This work was supported by the Finnish Pro Mare Balticum Walter and Andrée de Nottbeck Foundation, funding the Cladocera project for KVD ("Cladocera of the Baltic Sea – Taxonomy and Diversity") with fieldwork at Tvärminne Zoological Station, University of Helsinki, Finland.

**Keywords:** Baltic Sea; MinION; metabarcoding; invasives; ecology

# Shedding light on *Daphnia*: Interclonal differences in culture production at different light regimes

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*Daphnia* species possess circadian clock genes influencing their light sensitivity, behavior, feeding and growth and, therefore, affecting their production in culture. In our laboratory, two *Daphnia magna* clones (A and F) are routinely cultured for experimental investigations, prompting optimization of light conditions to enhance and sustain *Daphnia* production. Four illumination regimes (light: dark hours) were tested for this optimization: 12:12; 16:8; 24:0 (full-time light); and 0:24 (complete darkness, serving as a negative control). Parameters measured included age at first reproduction and total neonate production over three weeks. We found that the two clones exhibited differential productivity responses to light conditions, with clone A benefiting from the 12:12 regime and clone F from the 16:8 regime. These findings suggest potential variations in the genes governing circadian rhythms, contributing to the clone-specific nature of optimal light regimes for productive cultures. Such insights underscore the necessity for tailored adjustments for the culture conditions to accommodate these differences.

**Keywords:** *Daphnia magna* culture; interclonal variability; light regime; optimization of culture productivity

# Single cell transcriptome of adult *Daphnia magna*

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Full understanding of transcriptional responses to environmental and developmental cues is impossible without single cell (SC) resolution data. We performed a SC RNAseq survey of females and males of *Daphnia magna*, which is both a classic and emerging new model for eco-physiology, toxicology, and evolutionary genomics. We identified over 30 distinct cell types about half of which could be functionally annotated. First, we identified ovaries- and testis-related cell types by focusing on female- and male-specific clusters. Second, we compared markers between SC clusters and bulk RNAseq data on transcriptional profiles of early embryos, circulating hemocytes, midgut, heads, antennae II, and carapace. Finally, we matched *Daphnia* cell clusters with orthologous markers of 250+ cell types annotated in *Drosophila*. This allowed us to recognize striated myocytes, gut enterocytes, cuticular cells, 5 different neuron types, including photoreceptors, and 3 ovaries-related clusters, one of which tentatively identified as the germ line cells. We also tentatively identified circulating and non-circulating hemocytes. Numerous cell types remain unidentified, including those that map to FCA groups ambiguously or are characterized by *Daphnia*-specific markers. Likewise, many known or presumed cell types or tissues in *Daphnia* have not been identified to SC clusters. Paralogous members of protein families show a non-monotonous relationship between their amino acid divergence and transcriptional differences across cell clusters.

# The impact of *Microcystis aeruginosa* on *Daphnia magna* is shaped by ambient temperatures

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Along with increasing temperatures and eutrophication of lakes by nutrients, harmful algal blooms frequently challenge aquatic zooplankton organisms. Their poor food quality is due to lacking cholesterol and poly-unsaturated fatty acids. As ectothermic planktonic crustaceans show high metabolic rates, high growth rates and fast reproduction in warm conditions, their elevated demand for food must be met by high food ingestion rates at elevated temperatures. Accordingly, the detrimental impact of low-quality-food was assumed to increase corresponding to rising ambient temperatures. *Daphnia magna* were fed with the green algae *Desmodesmus subspicatus*, the cyanobacteria *Microcystis aeruginosa* or a mixture of both. Strains of *M. aeruginosa* free of microcystin but containing inhibitors of the digestive proteases chymotrypsin or trypsin (NIVA Cya 43 or PCC 7806) were used. Animals were acclimated at 10°C, 20°C and 30°C. Life table experiments documented impacts of the food regime and temperature conditions on growth and reproduction. The concentration of stored carbohydrates, lipids and proteins determined after 48 hours at various feeding conditions was affected by the ambient temperature as well as by the quality of the offered food. When only cyanobacteria were available, the energy status was comparable to starvation conditions. The energy reserves were depleted most strongly at warm temperatures. However, growth and reproduction were negatively affected by cyanobacteria also under cold conditions. The role of inhibition of the respective proteases will be discussed.

**Keywords:** cyanobacteria; harmful algal blooms; food quality; energy reserves; energy metabolism

# Urbanization drives adaptation to cyanobacterial blooms in zooplankton grazers

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Understanding how populations respond to urbanization is an important topic in contemporary evolutionary biology and has significant applied implications for biodiversity conservation. Eutrophication and subsequent cyanobacterial blooms due to rapid urbanization are reshaping aquatic ecosystems and altering biological evolution. In an integrated multivariate approach that addresses morphological and life history traits, we tested the hypothesis that aquatic grazers inhabiting more urbanized waters had become more tolerant to urban cyanobacterial blooms through adaptive evolution along the well-defined rural–urban gradients. We also quantified the relative importance of evolution of mean trait values, phenotypic plasticity, and evolution of phenotypic plasticity to total trait changes under urbanization using a reaction norm approach. We found that urban grazers have genetically adapted to urban cyanobacterial blooms, which is due primarily to selection for reduced cyanobacteria-induced phenotypic plasticity in urban eutrophic conditions. The evolution of plasticity and evolution of mean trait values played significant roles in mediating evolutionary change in multiple phenotypic traits. The plasticity played a significant role in mediating change in all traits except for offspring size. Our experiment provides strong support for contemporary adaptive evolution under urbanization, and highlighted the importance of quantifying genetic variation of phenotypic plasticity when evaluating drivers of life evolution in cities.

**Keywords:** urbanization; adaptive evolution; phenotypic plasticity; cyanobacterial blooms; *Ceriodaphnia cornuta*