Evolutionary toxicology: The acute and chronic effects of chemical exposure and climate change on the life history traits and gene expression of resurrected *Daphnia magna* clones in a multiple-stressor world

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Introduction

- Resurrected *Daphnia* as a model organism to investigate micro-evolutionary adaptions of natural populations to multiple environmental stressors
- **Resurrection ecology:** Diapausing eggs as indicators of historical lake and landscape development.
  - Through sexual reproduction, *Daphnia* produce sexual eggs (ephippia), which can persist as resting stages embedded in the sediment for centuries (Fig. 1) [1]
- **Evolutionary toxicology:** Examines the effects of environmental pollutants on genetics of modern and ancient *Daphnia magna* clones and their multi-generational and population-wide effects [2]
- ***Daphnia sp.*:** Keystone species with an important central role in the food web dynamics of lentic ecosystems. Model organism in ecology, ecotoxicology and biomedicine based on short generation time, sequenced genome and transparency [3]
- **Goal**: Based on this project, the morphological and molecular adaptation mechanisms of the genus *Daphnia* to multiple stressors in the form of climate change and chemical exposure will be considered at different levels of a multiscale approach over a period of several hundred years

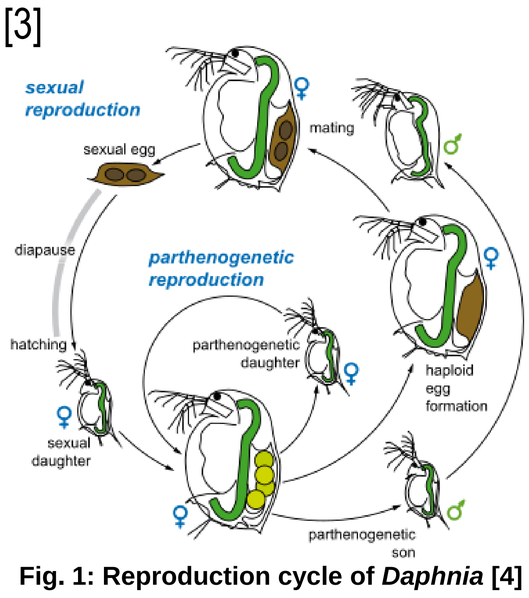
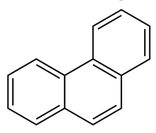


Fig. 1: Reproduction cycle of *Daphnia* [4]

Material & Methods

- Ephippia of historical *D. magna* clones were sampled at Lake Ring, Denmark, established by Orsini et al. [5] & at Stadtsee, Germany within the **Bad Waldsee project**
- Sexual eggs are resurrected according to protocol of Orsini et al. [6] and maintained in culture under optimal conditions
- *Daphnia* were exposed to phenanthrene (PHE), a model polycyclic aromatic hydrocarbon (PAH)



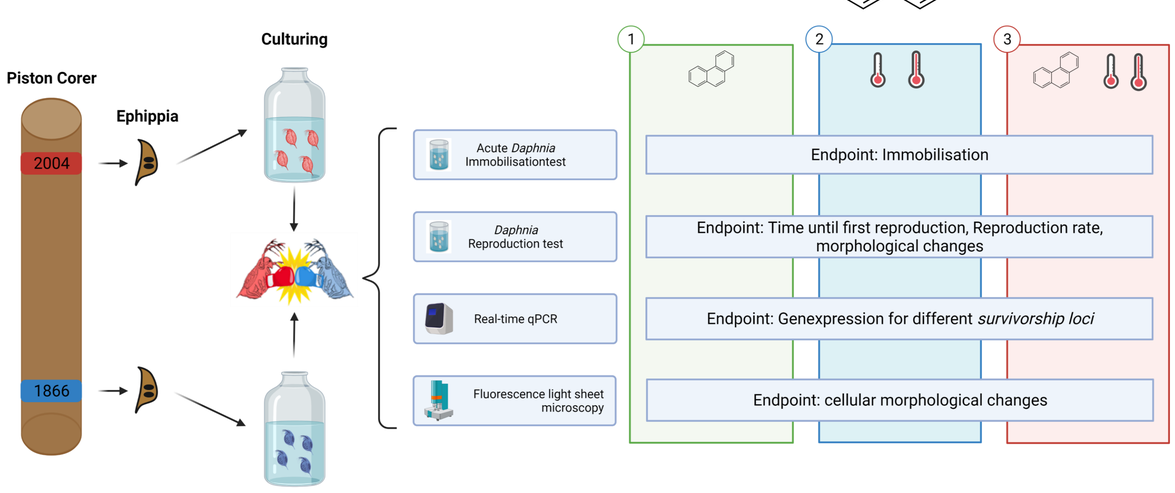


Fig. 2: Test strategy: Single stressors in the form of (1) chemical exposure, (2) temperature increase/ decrease and multiple stressors (3) with chemical exposure and temperature change

- According to OECD guidelines *Daphnia* were cultured at 20 ± 1°C, 16:8 h Light/Dark in M4 Medium
- The chronic effects according to OECD 211 [7] of two *D. magna* clones from Lake Ring, Denmark have already been investigated

Results & Discussion

- First results for chronic effects of modern *D. magna* clones (2_1 from 2004) and ancient clones (88_1 from 1866) from Lake Ring with phenanthrene exposure at 20 ± 1 °C

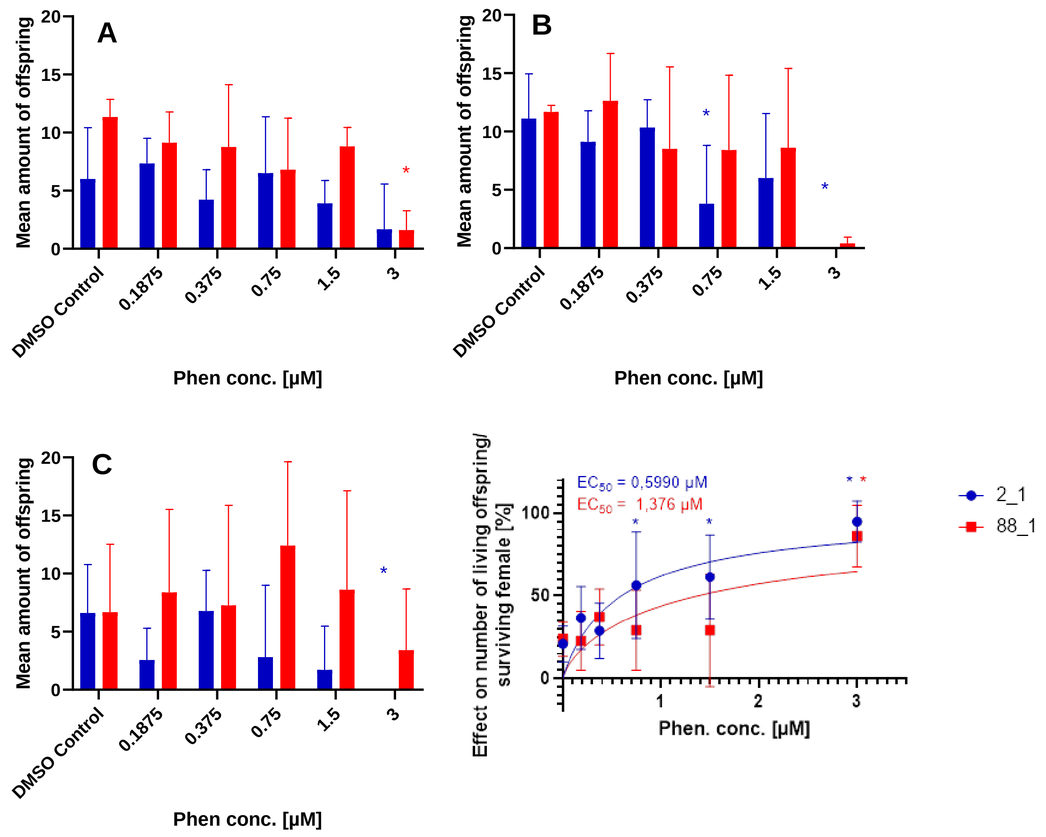


Fig. 4: Number of offspring for 1st (A), 2nd (B), and 3rd brood (C) and total number of living offspring in the 21-d Daphnia magna reproduction test at 20 ± 1 °C with PHE exposure (µM)

- Modern *D. magna* clones showed higher sensitivity in terms of their reproductive rate to chronic PHE exposure at 20 ± 1°C

Conclusion & Future plans

- In addition to the effects on reproductive rate with modern and ancient *D. magna* clones, data were also collected for reproductive time, morphology, and acute effects, which also showed stronger effects for modern *Daphnia* (data not shown)
- In addition to continuing the multiscale approach, more different aged *Daphnia* will be tested
- *Daphnia* will be examined in the future mainly from the sediments of the Stadtsee, Bad Waldsee, Germany within the DFG funded Bad Waldsee project
- **Bad Waldsee project:** An interdisciplinary research team of geoscientists, biologists, and historians was assembled to conduct high temporal resolution diatom and pollen analyses and geochemical-sedimentological investigations on existing sediment cores from the profundal of Stadtsee and to intersect and calibrate them with information from historical written records, deeds, charters, and maps
- This completeness of contemporary historical information, rarely found elsewhere, allows phenotypic differences of modern and ancient clones, such as different sensitivities to certain stressors, to be better discussed, represented, and placed in the overall ecological-anthropogenic context.
- First sediment samples were taken at the Stadtsee and are currently being processed

Project Website

References: **[1]** Burge, D. R., Edlund, M. B., & Frisch, D. (2018). Paleolimnology and resurrection ecology: The future of reconstructing the past. Evolutionary Applications; **[2]** Bickham, J. W. (2011). The four cornerstones of evolutionary toxicology. Ecotoxicology, 20(3), 497-502; **[3]** Seda, J., & Petrusek, A. (2011). Daphnia as a model organism in limnology and aquatic biology: introductory remarks. Journal of Limnology, 70(2), 337-344; **[4]** wiki image: https://commons.wikimedia. org/ wiki / File DaphniaMagna_ LifeCycle_DVizoso.svg; **[5]** Orsini L, Marshall H, Cuenca Cambronero M, Chaturvedi A, Thomas KW, Pfrender ME, Spanier KI, De Meester L. 2016. Temporal genetic stability in natural populations of the water flea Daphnia magna in response to strong selection pressure. Mol Eco 25: 6024 6038; **[6]** Cuenca Cambronero and Orsini. 2018. Resurrection of Dormant Daphnia magna : Protocol and Applications. J. Vis. Exp. (131); **[7]** OECD (2008). Guidelines for the Testing of Chemicals: 211 - Daphnia magna Reproduction Test. OECD ILibrary. https://doi.org/http://dx.doi.org/10.1787/9789264070127-en

This project has received funding from Excellence Initiative of the German federal and state governments - RWTH Postdoctoral Start-up grant (S. Crawford; StUpPD_351-18) & RobustNature Excellence Cluster Initiative & Bad Waldsee project