Paleoecotoxicology reveals higher tolerance of naive Daphnia magna genotypes to polycyclic aromatic hydrocarbons (PAH)

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Paleoecotoxicology enables the study of evolution of chemical tolerance [1]. The aim of the study is to investigate the **evolution of tolerance** to polycyclic aromatic hydrocarbons (PAH) in Daphnia magna in function of historical exposure to chemical stress
Resurrected populations of D. magna spanning a century, and including populations from semi-pristine and polluted environments were exposed to environmentally relevant concentrations (sediment) of PAH
Lake Ring: Shallow mixed lake, which was oligotrophic at the beginning of the 19th century, suffered from eutrophication from sewage inflow in the 1950-1960, suffered from increased land use from 1975 to ca. 2000 and partially recovered in modern times [2] (Fig. 2)
PAH: PAH are mainly formed by incomplete combustion or by the emergence of oil spills. Due to the low volatility, high sorption and lipophilicity of PAHs, they spread ubiquitously in the environment by binding to dust or soot in the air. Model PAH in this study was **Phenanthrene (PHE)**

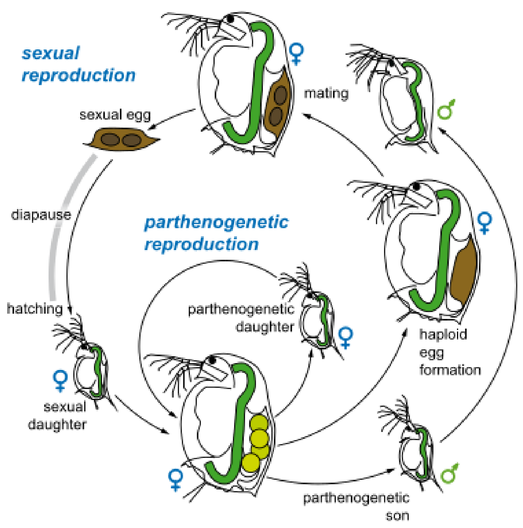
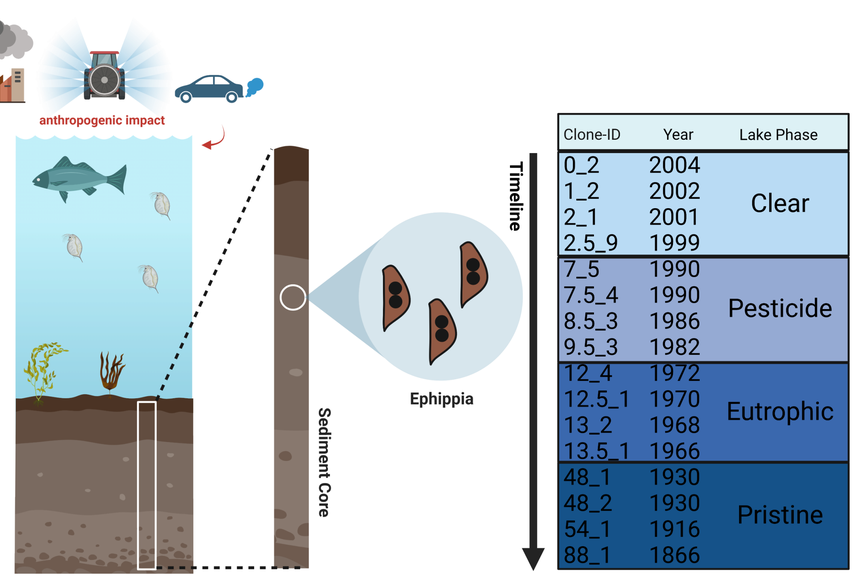
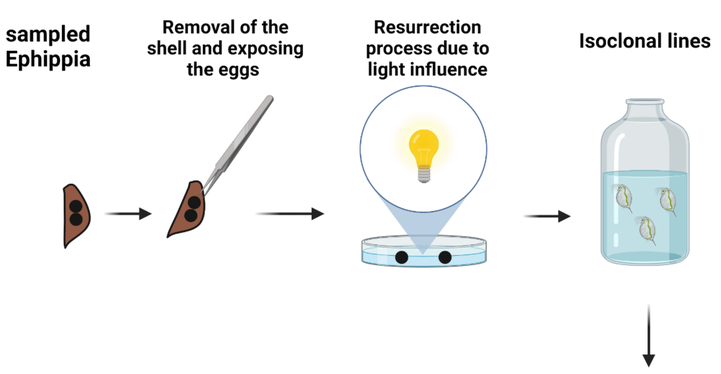


Fig. 1: Reproduction cycle of *Daphnia* [3]



Template adapted from: Dakota Holmes

Fig. 2: Sampling strategy Lake Ring (Jutland, Denmark)



Acute Daphnia Immobilisation Test:
Range finding for chronical exposure (EC_{10})

20°C

Daphnia magna Reproduction Test:
Endpoints: Time of primiparity, time of first and second brood, total number of offspring, morphological changes, size.

20°C

Sampling for RNA sequencing and Microbiome:
10 isoclonal Daphnia each sampled for RNA seq and Microbiome (Sampling of exposed Daphnia at the same developmental point before primiparity)

RNA Seq and Microbiome:
Analysis of differential gene expression and differential splicing of mRNAs.
Study of the microbiome composition.

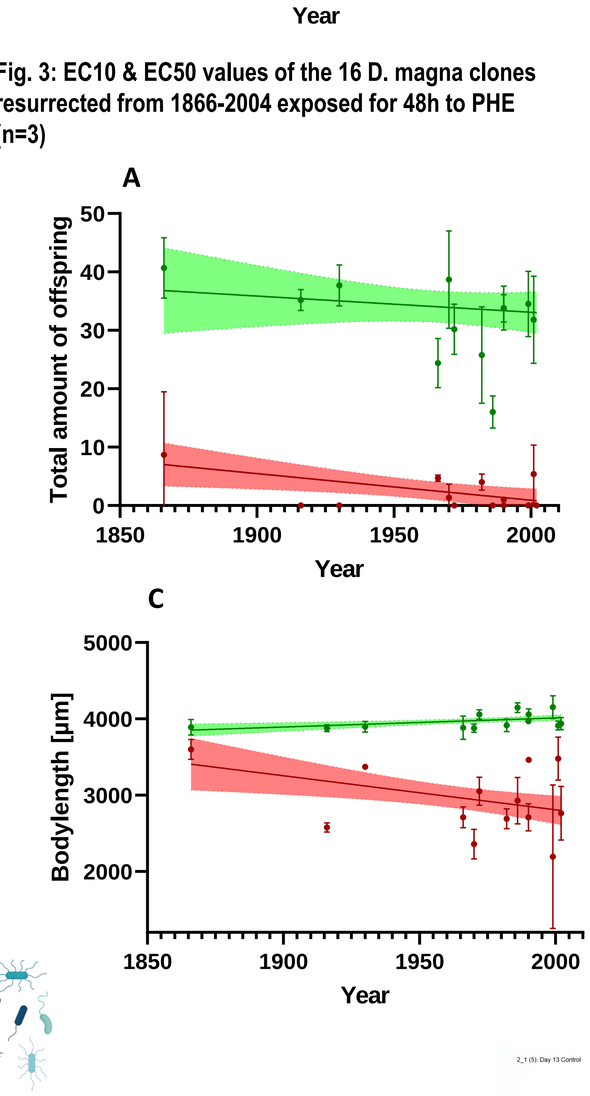


Fig. 3: EC10 & EC50 values of the 16 D. magna clones resurrected from 1866-2004 exposed for 48h to PHE (n=3)

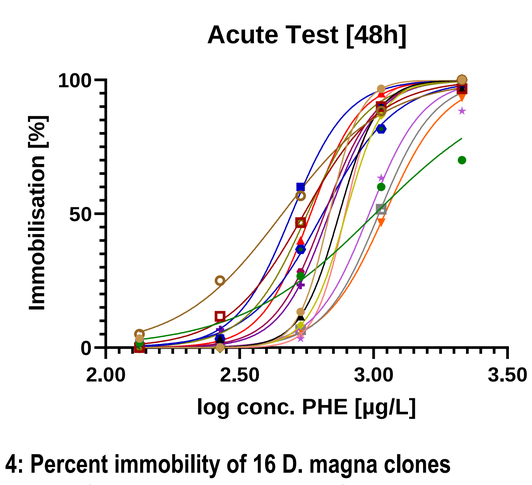


Fig. 4: Percent immobility of 16 D. magna clones resurrected from 1866-2004 exposed for 48 h to PHE (n=3)

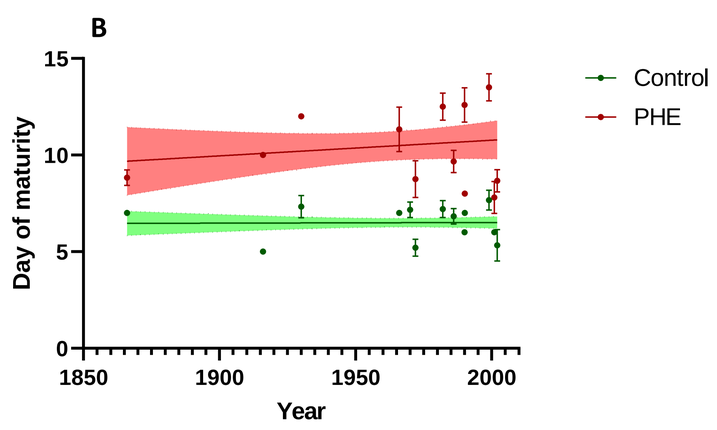


Fig. 5: Chronic effects on population level on Control and PHE exposed Daphnia: PHE caused reduce size at maturity, delayed maturation and lower fecundity [A] total number of offspring for 1_{st} and 2_{nd} brood; [B] Day of maturity; [C] length measurement of adult Daphnia after release of 2_{nd} brood.

Conclusion & Next steps

Prior exposure to chemical stress reduces tolerance to novel chemical stress (e.g. PAH).

Effect-based gene expression patterns will be investigated by performing RNA-sequencing.
It will also be investigated whether phenanthrene alters the functionality of the gut microbiota. A change in algae uptake by the exposed daphnia was already seen when the reproduction test was performed.

Further studies will be investigated with resurrected Daphnia from the Stadtsee (Bad Waldsee, Germany) (DFG funded Bad Waldsee project).

Fig. 6: Control Daphnia (left) and PHE exposed Daphnia (right) of clone 2_1 (top) and 7.5_4 (bottom)

Exposed daphnia from clear and pesticide phases show reproductive differences (delay in development of offspring in pesticide phase) in addition to size differences.

References: [1] Korosi, J. B., Thienpont, J. R., Smol, J. P., & Blais, J. M. (2017). Paleo-ecotoxicology: what can lake sediments tell us about ecosystem responses to environmental pollutants?. Environmental Science & Technology, 51(17), 9446-9457.; [2] Cuenca Cambronero, M., Marshall, H., De Meester, L., Davidson, T. A., Beckerman, A. P., & Orsini, L. (2018). Predictability of the impact of multiple stressors on the keystone species Daphnia. Scientific reports, 8(1), 17572.; [3] https://en.wikipedia.org/wiki/Daphnia#/media/File:DaphniaMagna_LifeCycle_DVizoso.svg;

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