Phenotypic and behavioural plasticity of *Daphnia magna* (Strauss) under salinity stress

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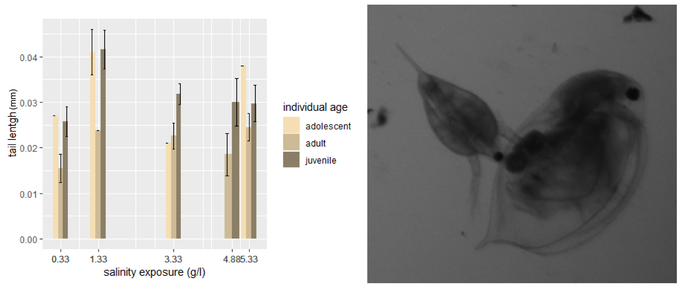
Background and Summary

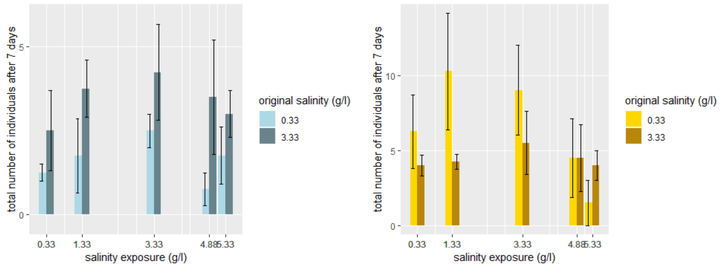
The over-salinisation of freshwaters is a global problem driven by climate change and chemical waste [9]. One of the means used to monitor changes in the quality of water is the use of bioindicators which are organisms with measureable sensitivity to contaminants and toxicants in the environment [3]. *Daphnia magna* is a promising parthenogenetic example which displays extreme phenotypic plasticity [10]. The causal relationship between the behavioural plasticity and metabolic changes in reaction to salinity stress in *D. magna* has been understudied, especially in terms of combining ontogenic, reproductive, behavioural, and metabolic responses [1, 2]. Here, we used two clones of *D. magna* to investigate the effects of environmental heterogeneity, with focus on water salinisation, on phenotypic and behavioural plasticity. This was done by measuring changes in population dynamics, tail length, pigmentation and swarming. Spectroscopy as a method in ecotoxicology has recently emerged as a method to investigate the stress response in *D. magna* [5]. Infrared spectroscopy was applied to characterise evidence of oxidative stress in an unstressed clone. Nuclear magnetic resonance was used to characterise a metabolic response in chronically stressed and unstressed clones. Our research supports the use of *D. magna* as a bioindicator with the potential to build databases of both measurable variables in toxic environments and the changing status of anthopogenic environmental heterogeneity.

Phenotypic plasticity and Behavious

Morphological changes suggest phenotypic plasticity at 4.88g/l NaCl and above. The NaCl concentration of the original habitat significantly affected the tail lengths ($F_{1,0} = 15.6295, P < 0.001$) while the age structure of the swarm had no significant effect on the tail length.



Population dynamics are linked to salinity exposure as well as original habitat. Populations from 0.33g/l NaClhabitats show higher survival in low salinities, if they started out as a swarm (right), while populations from3.33g/l NaCl habitats not forming swarms show higher survival in salinities of 3.33g/l and above (left).



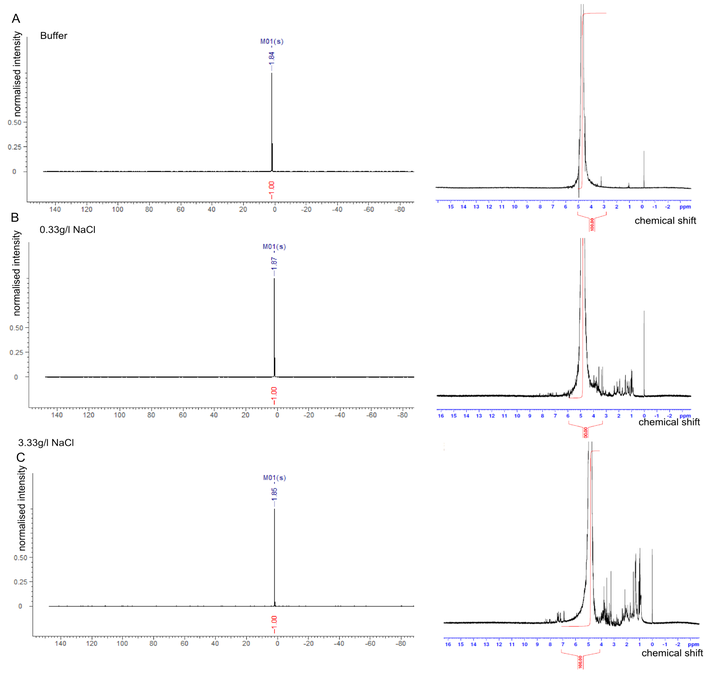
Individual behaviour is mainly dependent on swarm behaviour, but influenced by salinity and algae. Collisions between individual *D. magna* happened frequently in high salinity concentrations and swarms.

Methods

D. magna were kept in ADaM (**A**achener **Da**phnien **M**edium) after Klüttgen *et al.* (1994) [4]. Population of either 2 individuals (1 adult, 1 juvenile) or a swarm of 6 individuals (2 adults, 4 juveniles) were exposed to salinity stress varying from 0.33g/l to 5.33g/l during a 24h photo-period over a period of 15 weeks which each exposure lasting 7 days. Behavioural and phenotypic changes were measured through visual observation and light microscopy. Behaviour was quantified using stasis, rotation, and collision as binary variables. Following the observation, *D. magna* were lyophilised. A whole organism metabolome was measured using Nuclear Magnetic Resonance (NMR) and Fourier-transform infrared spectroscopy (FTIR). The NMR protocol was adapted from Nagato *et al.* 2013 and Kovacevic *et al.* 2016 using a buffer with 10mg/l TSP, 31.2g/l sodium phosphate dihydrate, 0.1% w/v sodium azide and 500μl D_2O [6, 5]. All data were analysis using R 3.5.3. For behaviour and population dynamics a Shapiro–Wilk test and generalised linear models were used. Individual behaviour was analysed using a binomial regression and an analysis of variance [8]. Imaging data was processed and analysed using Fiji ImageJ.NMR spectra were analysed using Spectrus Processor (version Advanced Chemistry Development, Inc., Toronto, On, Canada). Peaks were assigned to functional groups using Socrates 2004 [7].See report in the QR code for more details on the methods.

Metabolome

The metabolome shows a slight increase between treatments for populations from the 0.33g/l habitat. Both phosphorous and amino acid quantities are increased in *D. magna* from the 3.33g/l habitat. Seven amino acids were identified in proton NMR sprectra. Following Nagato et al. (2013), certain peaks in 2 are likely to be indicative of amino acids inD.magnawith valine at 1ppm, leucine at 1ppm, isoleucine at 1ppm, threonine at 1.29pmm, alanine at1.48ppm, arginine at 2ppm, methionine at 2.14ppm, glutamate at 2.3ppm, lysine at 3.24ppm, glycine at3.57pmm, and phenylalanine at 6.9ppm [6]. In the spectra obtained from the 0.33g/l culture, peaks werepresent at positions corresponding to leucine, valine, alanine, methionine, lysine, and glycine though allsignals were below 0.1kHz. Signals of the 3.33g/l exceeded an intensity of 5x10-5for all signals thatwould correspond to amino acids. However, the number of hydrogen atoms at the site of the peaksonly corresponded with the number of hydrogens for valine (0.98ppm), leucine (1.29ppm), or glycine(3.78ppm). A signal at 5.44ppm could not be assigned to any amino acid identified by Nagato et al.(2013), but is indicative of cysteine.



Conclusions

This study shows the effect of chronic exposure to salinity stress prior to changes in habitat salinity to affect the population dynamics and behaviour of population of *D. magna*. The metabolism of *D. magna* increases with chronic salinity exposure and populations with an enhanced metabolism have higher population sizes when they form swarms. Swarming behaviour is affected by population dynamics first and the effect of salinity exposure and total population size thereby indirectly influences the swarming behaviour. In cases of environmental heterogeneity, the plastic response of *D. magna* is thereby expected to be dependent on metabolism, diet, swarm formation, the salinity of the original habitat, and the exposure which manifest in phenotypic and behavioural plasticity. Future work needs to explore further molecular causes and swarming patterns.

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Contribution Statement

This poster is based on the work produced by AdSK as part of her project report for her BSc degree in Genetics. MSK provided experimental and statistical guidance. RH hosted and supervised the project. All authors produced the poster.

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