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BSc Genetics
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Metabolomic changes underlying phenotypic and behavioural plasticity of *Daphnia magna* (Strauss) in reaction to salinity stress

Abstract

The crustacean *Daphnia magna* is a highly plastic model organism in ecotoxicology. However, the causal relationship between the behavioural plasticity and metabolic changes in reaction to stress in *D. magna* has been understudied and many of the findings regarding the species have not been revisited as new insights on zooplankton behaviour, metabolism and morphology emerged. Here I show that salinity affects both phenotypic and behavioural plasticity in *D. magna* through modification to individual size, pigmentation and behaviour with a swarm. Furthermore, individual *D. magna* in small populations are shown to be physically harmed by their diet causing eutrophication over long time periods. Populations grown in habitats of specific salinities display differences in behavioural and phenotypic plasticity apparent in their swarming behaviour and population dynamics following an exposure. Slight salinity changes were confirmed to increase population sizes as population dynamics are affected by salinity and individual behaviour was shown to be affected more by the swarm structure than the population size. This confirms phenotypic and behavioural plasticity to cause a fitness increase in reaction to environmental heterogeneity. Amino acid expression differences and phosphate decrease suggest an increase energy production in a population stressed prior to exposure and contrast previous studies suggesting a decrease in stress situations. The lack of clear phenylalnine signals in the metabolome suggests alterations to hormones including dopamine. However, the findings do not agree with the previous suggestion that tail length is proportional to salinity stress and that survival in unchanged medium is proportional to salinity tolerance. While the importance of swarm structures to the display of behavioural plasticity in reaction to salinity exposure has been shown here, discrepancies between findings and literature demonstrate that *D. magna* metabolomics should be further investigated to investigate the mechanisms behind the phenotypic and behavioural plasticity of the species.

Introduction

In heterogeneous environments habitat changes occur for all existing populations, often causing stress situations (Balaguer et al. 2001, Valladares et al. 2014). Environmental heterogeneity affects a population's behaviour just as it affects the phenotype, often by triggering a plastic response (Gianoli & Valladares 2011, Palacio-López et al. 2015). In aquatic environments such heterogeneity is most commonly triggered by human influences (Brans et al. 2018). Reactions to environmental heterogeneity may be plastic in populations with a robust genotype (Gianoli & Valladares 2011). Phenotypic plasticity (Gause 1947) manifests in the phenotype either via the development of novel structures or via the atrophy or hypertrophy of existing structures (Bateson & Gluckman 2011). As phenotypic plasticity is closely linked to development it is reflected in a species' life history (West-Eberhard 2005). However, the exact effect of developmental noise on this is still unclear (Yampolsky & Scheiner 1994). Development is affected as maternal stress including malnutrition and may trigger a plastic response in the offspring (Bateson & Gluckman 2011).

Daphnia magna

The aquatic Crustacean *Daphnia magna* is a model organisms for phenotypic plasticity and ecotoxicology as the species is easily maintained in laboratory conditions (Adema 1978, Asselman, De Coninck, Pfrender & De Schamphelaere 2016, Latta et al. 2012). As the species is directly affected by humans through environmental poisoning, it has been established as a biomarker for environmental heterogeneity in aquatic habitats (Brans et al. 2018). While a genome of *D. magna* has been acquired and the proteome and the epigenome of the species both within and outside of stress situations have been described, the *D. magna* metabolome remains to be explored further (Latta et al. 2012, Routtu et al. 2010). Populations of *D. magna* can stabilise, grow or crash in stress situations. A possible reaction to moderate stress situations is an increase in offspring number (Khudr et al. 2018). Young *D. magna* respond to stress better than the adults (Schumpert et al. 2014). Populations of *D. magna* in environments with slightly higher salinities have been shown to produce more offspring than populations in natural environments (Arnér & Koivisto 1993). Chronic toxicity affects a population of *D. magna* over several generations (Adema 1978). *D. magna* is a mainly parthenogenic species, though a sexual reproduction cycle can be triggered by severe stress situations. In parthenogenic species like *D. magna*, genotype is conserved within the population which then enables a bigger range of plasticity within a population of *D. magna* (Yampolsky & Scheiner 1994).

Phenotypic plasticity

Phenotypic plasticity has been defined as different phenotypes being produced by the same genotype (Gotthard & Nylin 1995). If the costs of plasticity are too high, a population will crash (DeWitt 1998). Further limits to plasticity include ontogeny which may be affected directly by the environment, e.g. through injury (Gianoli & Valladares 2011). However, most freshwater organisms have a plastic response to environmental mismatches such as changes in water pollution (Arnér & Koivisto 1993). In *D. magna* phenotypic plasticity has been associated with changes in life history and morphology of the exoskeleton, especially in reaction to kairomones which are predator-borne chemical cues (Lynch 1980). *Daphnia magna* morphology is also affected by temperature, displaying cyclomorphosis which cause morphological changes across temporal cycles in form of a dorsoanterior expansion of the head and the tail (Hebert 1978). Warren (1900) suggested a linear correlation between tail length, age and salinity stress. Carapace length was shown to be decreased in situations of salinity stress (Arnér & Koivisto 1993).

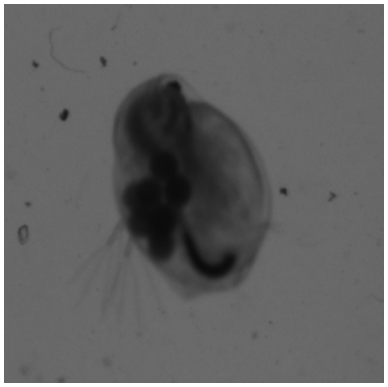

Figure 1: **A *D. magna* adult with eggs**
Healthy adults of *D, magna* are transparent apart from their compound eye, egg pouch and. Amongst over 50 species of *Daphnia* that compete with other and other filter-feeders, *D. magna* is one of the largest species (Hebert 1978, Repka et al. 1999). gut.

Molecular sources of plasticity

Sources of phenotypic plasticity in *D. magna* have mainly been identified as changes in global DNA methylation, alternative splicing and protein phosphorylation as well as acetylation (Asselman et al. 2015, Latta et al. 2012). Cysteine CpG methylation in *D. magna* drives cell development and gene regulation (Asselman, De Coninck, Pfrender & De Schamphelaere 2016). Such hypermethylation has been shown to be transmitted accross generations (Jeremias et al. 2018). CpG hypermethylation causes phenotypic plasticity via alternative splicing (Price et al. 2018).

Heat shock proteins are involved in the stress response of *D. magna*. Changes in HSP60 and HSP70 expression have been quantified during stress triggering phenotypic plasticity (Mikulski et al. 2009, Pauwels et al. 2007, Schumpert et al. 2014). HSP60, which regulates mitochondrial protein transport and DNA metabolism is slightly smaller in *D. magna* than in humans with only 50 to 56kb (Pauwels et al. 2007). Levels increase in response to kairomones (Mikulski et al. 2009). Hsp70 which regulated protein stability and prevention of cell death in *D. magna* is enhanced under stress, especially thermal stress (Mikulski et al. 2009, Schumpert et al. 2014). The *D. magna* HSP70 is highly homologous to *Caenorhabditis elegans* and *Drosophila melanogaster* Hsp70 (Schumpert et al. 2014).

Another form of transcriptome variation in *D. magna* during salinity stress is alternative splicing. Alternative splicing in *D. magna* phenotypic plasticity has been found in genes associated with osmoprotectant biosynthesis, kairomone response, chitin metabolism, and ion transport (Latta et al. 2012).

Fluctuations in ionic concentrations and temperature rises in the *D. magna* habitat have been shown to affect the growth rate of individuals with atrophy resulting in a thrifty phenotype (Adema 1978). Extreme environmental heterogeneity triggers an alternative sexual life cycle involving the production of males and fertilisation of haploid eggs (Stollewerk 2010). Physical damage to *D. magna* has been shown to trigger plastic trait variations in form of developmental noise (Yampolsky & Scheiner 1994). Further effects to the life history include a shift in the age structure of a population in response to scarceness of food which increases individuality (Preuss et al. 2008, Tsimring 2014). *Daphnia magna* are generalists

that can survive vast environmental heterogeneity (Wikan & Mjølhus 1996). Populations are able to return to an auto-regulated reproductive equilibrium due to their resilience which Pimm (1984) defined as determining 'the persistence of relationships within a system'. Such persistence is reflected in the fitness of *D. magna* populations following the evolutionary synthesis as they are able to anticipate future plastic responses needed (Bateson & Gluckman 2011). Selection is enhanced by the polyphenism phenotypic plasticity may cause (West-Eberhard 2005). Behaviour, often regarded as a form of phenotype, has also been suggested as a part of a plastic response, thereby increasing fitness (Scheiner 1993).

Swarming in *D. magna*

The behavioural plasticity of *D. magna* has been studied less than phenotypic plasticity, but plasticity and stress have been linked (Latta et al. 2012). Plasticity in *D. magna* also affects behaviour. Behavioural plasticity only present in presence of a trigger like e.g. salinity stress is activational (Snell-Rood 2013). Like with other zooplankton species, the migration of *D. magna* is dependent on light and temperature (Nurminen et al. 2007, Ringelberg 1964). As a heliotrophic species, *D. magna* behaviour is dependent on the light in the medium which is influenced by the phytoplankton content (Ringelberg 1964, Warren 1900). *D. magna* form swarms that consist of 6 or more individuals. The density of a swarm which is directly affected by the environment is an important factor in stress resulting from food competition (Guckenheimer et al. 1977). The intrinsic dynamics of a swarm are limited by crowding as their movement is forced by extrinsic trends like the light conditions of the medium or its salinity content as well as fluctuations in population dynamics (Preuss et al. 2008). A swarm of *D. magna* is a non-linear system and will therefore display statistical movement for which an individual swimming pattern following Brownian motion is characteristic (Eckmann et al. 1987). All individuals within a swarm move in relation to each other and their movement is reflective of the overall (Mach & Schweitzer 2007, Ringelberg 1964). *Daphnia magna* swarms generally form into a vortex structure that dissolves during stress situations (Hsin 2006). Individual behaviour during stress situations commonly includes spinning where an individual rotates either on the spot or by tumbling around within the swarm, often colliding with other individuals (Dodson et al. 1995).

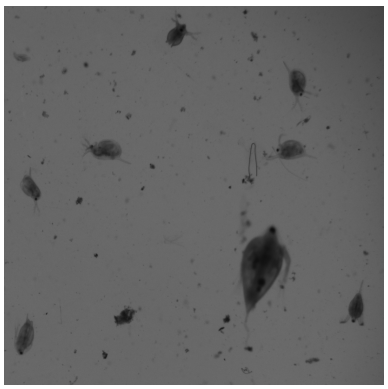

Figure 2: ***D. magna* form swarms when a population exceeds 6 individuals**
A swarm of *D. magna* trained in 3.33g/l NaCl in Aachener Daphnia Medium (ADaM) exposed to a NaCl concentration of 3.33g/l does not show the typical vortex structure.

Previous models of *D. magna* swarms postulated that swarming is directly dependent on a swarm's age structure rather than the total population size (Mach & Schweitzer 2007, Ottermanns et al. 2014).

The *D. magna* metabolome

The plastic response that *D. magna* shows in reaction to salinity stress is reflected in the metabolome. The Cl^- from the saline habitat alters the osmoregularity of the *D. magna* (Mount et al. 1997). Due to the energetic costs of plasticity, a metabolomic approach provides a more comprehensive angle to plasticity and an understanding of the relationship between environment, phenotype and behaviour. The metabolism of *D. magna* is limited by the oxgen/nitrogen ratio of the environment and haemoglobin is upregulated (Arnér & Koivisto 1993, Yampolsky et al. 2014). Amino acid production has been associated with metabolome alterations in stress situations. An increase in phenylalanine in *D. magna* is indicative

of hormonal changes, especially alterations to dopamine and octopamine (Nagato et al. 2013). The increased energy production coming with a higher metabolomic rate has furthermore been associated with sub-lethal stress in *D. magna* (Kovacevic et al. 2016).

Hypothesis

Salinity stress in heterogeneous environments increases the metabolic rate through phosphorylation, methylation and alternative splicing, resulting in behavioural and phenotypic plasticity, manifesting in non-vortex swarming with individual distress and thrifty phenotypes with increased pigmentation.

Aims and Objectives

The objective of the project are to (I) determine the nature of swarming as a plastic behavioural train in *D. magna*, (II) establish a connection between whole organism metabolomics and epigenetic changes including alternative splicing, methylation and phosphorylation, (III) show how increases in salinity (g/l NaCL) as environmental heterogeneity trigger phenotypic and behavioural plasticity, (IV) demonstrate a different plastic response to salinity in populations from different habitats, and (V) show that salinity stress is not proportional to *D. magna* tail length and survival in unchanged medium is not proportional to salinity tolerance as concluded in Warren (1900).

Here, behavioural and metabolomic changes reaction to exposure to various concentration of NaCl were measured using bioimaging, FTIR, and NMR in order to obtain new insights into the stress reaction of *D. magna* and possible molecular causes of the observed plastic response.

Impact

As *D. magna* is an important model organism in ecotoxicology, it's stress response should be examined as closely as possible in order to understand the human effect on the environment better. Here, the stress reaction to chronic salinity stress is regarded under consideration of the effect that previous chronic salinity stress has which does not confirm previous findings from the literature. The plasticity of the *D. magna* stress response has phenotypic as well as behavioural aspects. Population dynamics and swarming behaviour affect each other and have shown to be plastic due to metabolome changes. Measurement of such metabolome changes further enable the usage of *D. magna* as a bioindicator. Here, the usefulness of NMR in metabolome studies of *D. magna* is demonstrated and FTIR is introduced as a new method for measuring metabolome changes.

Methods

Study system

Pathenogenic *D. magna* were used to explore population dynamics, swarming behaviour and metabolome changes in order to quantify a plastic response to salinity stress. The LC_{50} for *D. magna* is $7.8\mathrm{g}\mathrm{l}^{-1}$ (Bezirci et al. 2012). *Daphnia magna* (Fisher Scientific) were kept in ADaM (**A**achener **Da**phnien **M**edium) after Klüttgen et al. (1994) with salinities of 0.33g/l to 5.33g/l and considered trained after a continuous exposure of two weeks. The feed consisted of a mixture containing 1g/l *Chlorella spp.* powder (Naturya), 1g/l dried *Saccaromyces cerevisiae* (Allinson), 1g/l *Spirulina spp.* powder (Naturya), and 1g/l soy flour (Infinity Foods).

The *D. magna* exposed to different salinity stresses were grown in habitats of 0.33g/l and of 3.33g/l NaCl. To avoid the diurnal migraton caused by the Daphnia's circadian rhythm as previously described in Ringelberg (1964), *D. magna* were kept in a 24h photo-period.

Algae pollution

In order to identify the effect of light on a populations survival, 5 beakers with 10 individuals from each stock culture were kept in ADaM until all individuals were dead. They were fed every two days and the medium was not changed in order to test the effect of the medium changing to a green colour on the survival of the population as proposed by Warren (1900).

Population dynamics and Swarming Behaviour

As *D. magna* asexually produce clonal offspring, REvoSim (Garwood et al. 2019) was used to simulate possible changes to the coding and non-coding genome respectively throughout the behavioural assays during which the population went through 15 generations. The chance of mutation was set to 5 as Eberle et al. (2018) concluded *D. magna* to have a mutation bias of $\tilde{5}$ for behavioural genes.

To measure population dynamics, *D. magna* from the 0.33g/l NaCl and 3.33g/l NaCl stock cultures were exposed to NaCl concentrations of 0.33g/l, 1.33g/l, 2.88g/l, 3.33g/l, 4.88g/l and 5.33g/l. 4 repeats of either 2 individuals (1 adult, 1 juvenile) or a swarm of 6 individuals (2 adults, 4 juveniles) being exposed were performed over a period of 15 weeks. Each exposure lasted 7 days with feeding 1ml of the feed that the cultures received upon initial exposure, after 3 days, after 5 days and after 7 days.

A swarm of *D. magna* constitutes of 6 or more individuals within a population (Ottermanns et al. 2014). The mathematmodel generated from Erdmann et al. (2004), Mach & Schweitzer (2007) and Ottermanns et al. (2014) (see appendix for derivations) to predict vortex formation uses Langevin equations of the balance of forces describing individua *D. magna* to model the movement of an individual with

$$m\delta_t\vec{v} = -\gamma_0\vec{v} + de(t)\vec{v} - \Delta U(r) + \sqrt{2D}\vec{\xi}(t) \tag{1}$$

where $m\delta_t\vec{v}$ is the mass dependent movement, $-\gamma_0\vec{v}$ the noist disspation of the *D. magna* in the environment, $de(t)\vec{v}$ the movement itself, $de(t)\vec{v}$ the external potential energy considering helitrophism, and $\sqrt{2D}\vec{\xi}(t)$ a random force. This allows to determine the ensemble of forces acting as

$$m\delta_t\vec{v}_1 = -\gamma_0\vec{v}_i + [d_0 + \nu(t)]e_i(t)\vec{v}_i - \kappa_h[\vec{r}_i - \frac{1}{N}\sum_j \vec{r}_j] + \sqrt{2D}\vec{\xi}(t)] \tag{2}$$

where κ_h is the coupling constant that allows $de(t)\vec{v}$ and $de(t)\vec{v}$ to be substituted with specifications of the individual that suggest internal swarm interactions to have a stronger effect than internal noise using the velocity (ν) and energy (e) of the individual. For the overall swarm movement, this has the consequence that the Oseen law (Oseen 1910) which describe the flow of the viscous environement at low Reynold numbers allows to add a vector of $\vec{r}_i - \frac{1}{N}\sum j\vec{r}_j$ showing considering the Euclidian distance between agents r_{ij} which is determined by the distances between individuals r_i and r_j in order to determine movement within a swam as

$$m\delta_t\vec{v}_i = -\gamma_0\vec{v}_i + \kappa_F\vec{v}_F - \kappa_h[\vec{r}_i - \frac{1}{N}\sum j\vec{r}_j] + \sqrt{2D}\vec{\xi}(t) \tag{3}$$

where $\kappa_F\vec{v}_F$ is a force generated by all individuals. The consequence for the flow field ($\sum_{j\neq i}$) for the swarm movement is that

$$\vec{v}_F(\vec{r}_j) = \sum_{j\neq i}[\frac{R}{r_{ij}}\vec{v}_j + \frac{\vec{r}_{ij}\otimes\vec{r}_{ij}}{r_{ij}^2}]valid for r_{ij} \gg R \tag{4}$$

where R is an effective hydrodynamic radius. The flow field causes the entire swarm swimming into one direction when swimming closely which avoids collision.

Behaviour was measured with three binary variables: stasis, rotation and collision. Stasis was defined as a lack of diurnal migration and the failure to move away from a location within the medium. An organism that rotated on the spot was still considered stasis. Rotation was defined as an individual spinning or rapidly swimming in multiple circles. Collisions were measured both for individuals colliding with each other and with the edges of the beakers. Data was collected on the final day to heighten the likelihood of behaviour resulting from chronic salinity stress rather than from the transfer into a new environment. Possible combination of individuals displaying combinations of the measured binary traits were quantified. A rotation individual that was not static was tumbling around and collisions could either be between two moving individuals or one individual swimming into another during with both, either or none could be rotating.

Bioimaging

Movement was filmed for the 3 repeats of populations from 3.33g/l habitat with initial population size 2 individuals and from the 0.33g/l habtat with initial population size 6 for NaCl treatments of 0.33g/l, 1.33g/l, 3.33g/l, 4.88g/l and 5.33g/l in order to determine tail length. Images were collected on a Leica M205 FA upright Stereomicroscope using a 0.02 PlanAPO objective at the equivalent of 0.49 magnification and captured using a DFC365 FX (Leica) camera through the LAS AF v3.1.0.8587 software (Leica). The imaging allowed to measure stasis, a fourth binary factor.

Whole organism metabolomics

A whole organisms metabolome was measured using NMR and FTIR in order to identify changes in functional groups as evidence of the proteome changes found in Latta et al. (2012) and Asselman, De Coninck, Pfrender & De Schamphelaere (2016). The effect of diet was not tested directly, but studies from the GenToxLab in Ghent (e.g. Asselman et al. (2015)) performed similar population experiments on cultures that were fed a different diet. Metabolomic changes as shown in Kovacevic et al. (2016) and Nagato et al. (2013) were also analysed for the trained populations.

100 *D. magna* each from the stock culture grown in 0.33g/l NaCl and 2.88g/l NaCl were analysed by Fourier-transform infrared spectroscopy (FTIR) in The Mill, The University of Manchester, M1 3AL. *D. magna* were killed by being frozen at -80°C as spectra of *D. magna* at -20°C showed CO_2 peaks indicative of oxidative stress. 1 repeat of all remaining 5 exposures was also analysed. In preparation for the analysis, the samples were lyophilised for 1h at -20°C in order to allow for a higher resolution of the spectra. FTIR data was obtained using a Nicolet IS10FT-IR spectrometer (Thermo Scientific) with a temperature range of 0-70. Before analysis, all spectra were measured against a background of ADaM and collected in the region of 4000 – 650 cm-1 by co-adding 16 scans and at resolution of 4 cm^{-1} using the software OPUS to acquire an IR spectrum analysed for qualitative changes with special regard to carbon-containing functional groups. NMR spectra were analysed using Spectrus Processor (version Advanced Chemistry Development, Inc., Toronto, On, Canada). Peaks were assigned to functional groups using Socrates (2004).

The FTIR for the samples containing 100 individuals was followed by Nuclear magnetic resonance (NMR). Samples were expected to be in the same state as following the lyophilisation, since FTIR is non-invasive (Rosi et al. 2009). NMR samples were prepared with a protocol adapted from Nagato et al. (2013) and Kovacevic et al. (2016). Due to the composition of the ADaM solution, TMS which is hydrophobic could not be used for the buffer, so TSP was used instead. Samples were supplemented with 500µl of a buffer containing 10mg/l TSP, 31.2g/l sodium phosphate dihydrate, 0.1% w/v sodium azide and 500µl D_2O. NMR spectra were generated for protons with water exclusion and for phosphorous(31P). Samples were vortexed for 45s followed by 1 minute sonication at a 1% cycle to break the *D. magna* exoskeleton. Then samples were centrifugates at 4°C for 20 minutes at 12,000 rpm. The supernatant was then centrifugated with the same protocol. The following supernatant was transferred into a NMR tube. The NMR spectra for a water supplementation proton spectrum and a 31P spectrum were obtained using a Brucker Avance III 400 MHz NMR spectrometer. The machine was equipped with 4 mm double resonance MAS (15 kHz) with an actively shielded Z gradient at the the Manchester Institute of Biotechnology, The University of Manchester, M1 7DN. Tetramethylsilane (TMS) was used as the reference at a chemical shift of 0 ppm. 15N and 13C spectra were not obtained due to a lack of sensitivity for whole organism metabolomics. NMR spectra were analysed using Spectrus Processor (version Advanced Chemistry Development, Inc., Toronto, On, Canada) for qualitative changes with special regard to amino acids and phosphorylation content. Amino acids were assigned considering hydrogen numbers of the zwitterions and assignments in Nagato et al. (2013).

Statistical analyses

Both the population dynamics and the individual behaviour were analysed using R, version 3.5.3 (Great Truth), in RStudio (RStudio Team 2015). After running shapiro tests, the results of the tail length measurements, the swarm-specific population dynamics, and the individual behaviour were identified to be non-normal which was why a quasi-poission distribution was applied in all statistical tests.

Population dynamics were determined using the original training salinity, the exposure levels, and whether the total population in the end was a swarm or not as explanatory variables and the total population after exposure as well as the population growth rate. For the analysis, a generalised linear model (glm) with quasi-Poisson distribution, using the packages tidyverse (Wickham 2017), car (Fox & Weisberg 2011), and multcomp (Hothorn et al. 2008) with the original population size, whether the population was a swarm or not, the training salinity, and the exposure level were used as predictors. This was followed by an Anova which requires car (Fox & Weisberg 2011) in order to show the main effects. The summary of the glm provides the significance of the factors comprising each effect.

The individual behaviour was analysed using a binomial regression using the package nnet (Venables & Ripley 2002) with whether the population was a swarm or not, the initial population size, the age class of the organism, and the habitat salinity followed by an ANOVA in order to show the main effects. Imaging data was processed and analysed using Fiji ImageJ and the results of the imaging and observations

were analysed using R. Individual behaviour was analysed by applying a non-parametric test to the binary behavioural data using the training salinity and the salinity treatment as predictors.

Results

Morphological changes suggest phenotypic plasticity at 4.88g/l NaCl and above

For 4.88g/l and 5.33g/l NaCl, pigmentation in all individuals was increased and spread to the previously transparent parts of the *D. magna*. A thrifty phenotype as described in Gluckman & Hanson (2004) was observed in the bioimaging results for individuals from the 3.33g/l.

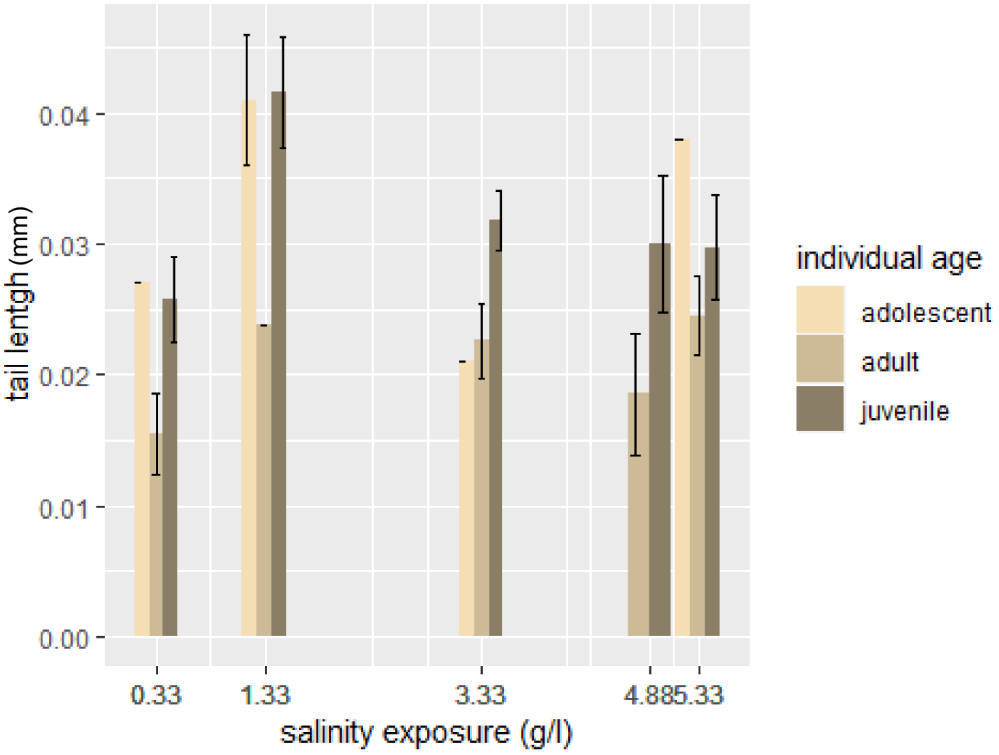

Figure 3: **Average tail lengths of 93 individual *D. magna* exposed to different salinity levels**

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.

Survival in unchanged medium is independent of the original habitat

Both cultures showed mean survival times of 26 days. Two populations in 0.33g/l NaCl had an increased reproduction rate and therefore survived for 41 days which was when all the medium in the beaker had evaporated, while one swarm survived for 10 days and two swarms survived for 20 days. *D. magna* grown in a habitat with 3.33g/l salinity survived for 20 to 30 days with one population surviving for 20 days, three populations surviving for 27 days and one population surviving for 30 days.

Population dynamics are linked to salinity exposure as well as original habitat

The REvoSim simulation showed no expected genome changes due to the low number of generations during the overall duration of the assays.

The effect of the salinity exposure on the total population size after 7 days was significant ($F_{(1,23)} = 9.7473, P < 0.01$). The initial population size ($F_{(1,16)} = 6.8518 P < 0.1$) and the interaction between the salinity of the training medium and the salinity exposure during the 7 days ($F_{(1,9)} = 3.9707 P < 0.1$) were

also significant. For the swarms, the highest survival was amongst the populations from the 3.33g/l habitats in 3.33g/l and 4.88g/l.

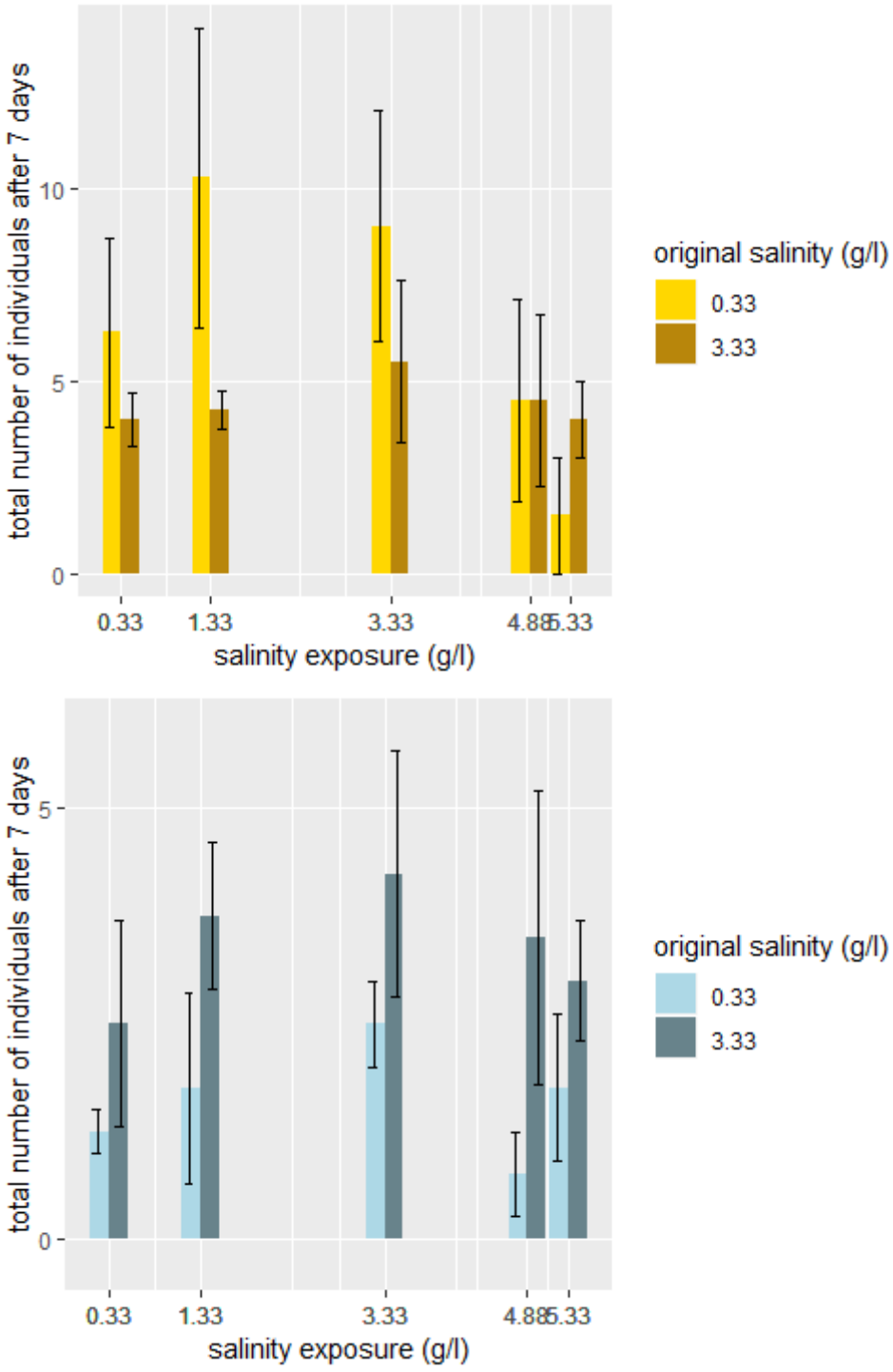

Figure 4: **Population dynamics show different reactions to salinity for different original swam sizes.**
The total population can be linked to the salinity stress using the initial population size, the presence of a swarm in the end of the experiments, the training concentration, the experimental treatment and the relationship between the treatment concentration and the exposure level. Populations from 0.33g/l NaCl habitats show higher survival in low salinities, if they started out as a swarm (A), while populations from 3.33g/l NaCl habitats not forming swarms show higher survival in salinities of 3.33g/l and above (B)

Individual behaviour is mainly dependent on swarm behaviour, but influenced by salinity and algae

Individual behaviour could be observed to include *D. magna* rotating and colliding as well as swimming towards light sources, regardless of whether they collided with the beaker walls in the process. While individuals clustered, vortex formations were not observed as swarms did not rotate in one direction in cases of swimming closely.

In high salt concentrations individuals were more likely to collide. Adults avoided each other in 0.33g/l by swimming above one another, whereas individuals in higher concentrations either did not avoid collisions or swam past each other.

Movement towards the light was observed in all salinities with one individual from the 0.33g/l NaCl habitat immediately turning around in order to swim towards the light. Some adults from both habitats appeared to be pushing against the ground with their antennae. This was mostly observed in habitats with increased algae growth. The *D. magna* from the 3.33g/l habitat kept on getting stuck on the algae when exposed to lower salinities.

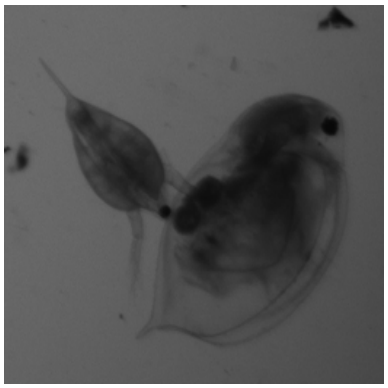

Figure 5: ***D. magna* colliding**
Collisions between individual *Daphnia* happened frequently in high salinity concentrations and swarms. 28 individuals collided either with other individuals or the beaker during the exposures.

There were 69 rotations and 31 cases of stasis amongst individuals. It was more significant to the individual behaviour whether the individuals were part of a swarm or not ($\chi^2 = 25.27, \mathrm{Df} = 7, \mathrm{p} < 0.001$) than the salinity exposure ($\chi^2 = 53.57, \mathrm{Df} = 28, \mathrm{p} < 0.01$). Furthermore, both the initial size of the swarm $\chi^2 = 12.1, \mathrm{Df} = 7, \mathrm{p} < 0.1$) and the age of the individuals ($\chi^2 = 25.26, \mathrm{Df} = 14, \mathrm{p} < 0.1$) showed slight effects.

The metabolism shows a slight increase between treatments for populations from the 0.33g/l habitat

The FTIR only gave results for population trained in 0.33g/l NaCl with treatments 0.33g/l NaCl, 1.33g/l NaCl, 3.33g/l NaCl, and 4.88g/l NaCl. Peaks at positions from 403.72cm^{-1} to 477.01cm^{-1} were considered to be in the fingerprint region of the spectra. All peaks in the fingerprint region were classified as very weak. The FTIR spectrum of the stock culture in 1.33g/l NaCl showed a peak of 1.113 at 1419.46cm^{-1} and was the only spectrum to show a peak in that region. Very strong peaks in the region from 1962.04cm^{-1} to 1971.04cm^{-1} indicate the presence of alkenes in the *D. magna* metabolome. A very strong peak with intensity 1.031 to 1.033 at 2160.04cm^{-1} to 2163.9cm^{-1} suggests alkyne. At 2359.33cm^{-1} to 2365.76cm^{-1} a very strong peak with at CO^2. The peaks at 2390.21cm^{-1} to 3300.49cm^{-1} with intensity 2.032 to 2.061 indicate OH or NH was strong for 1.33g/l treatment and medium strong for all other treatment. A strong peak of intensity 1.444 to 1.452 at 1634.18cm^{-1} for all treatments indicates C=0. Intensities were minimally increased for all relevant wavelengths as salinity increased.

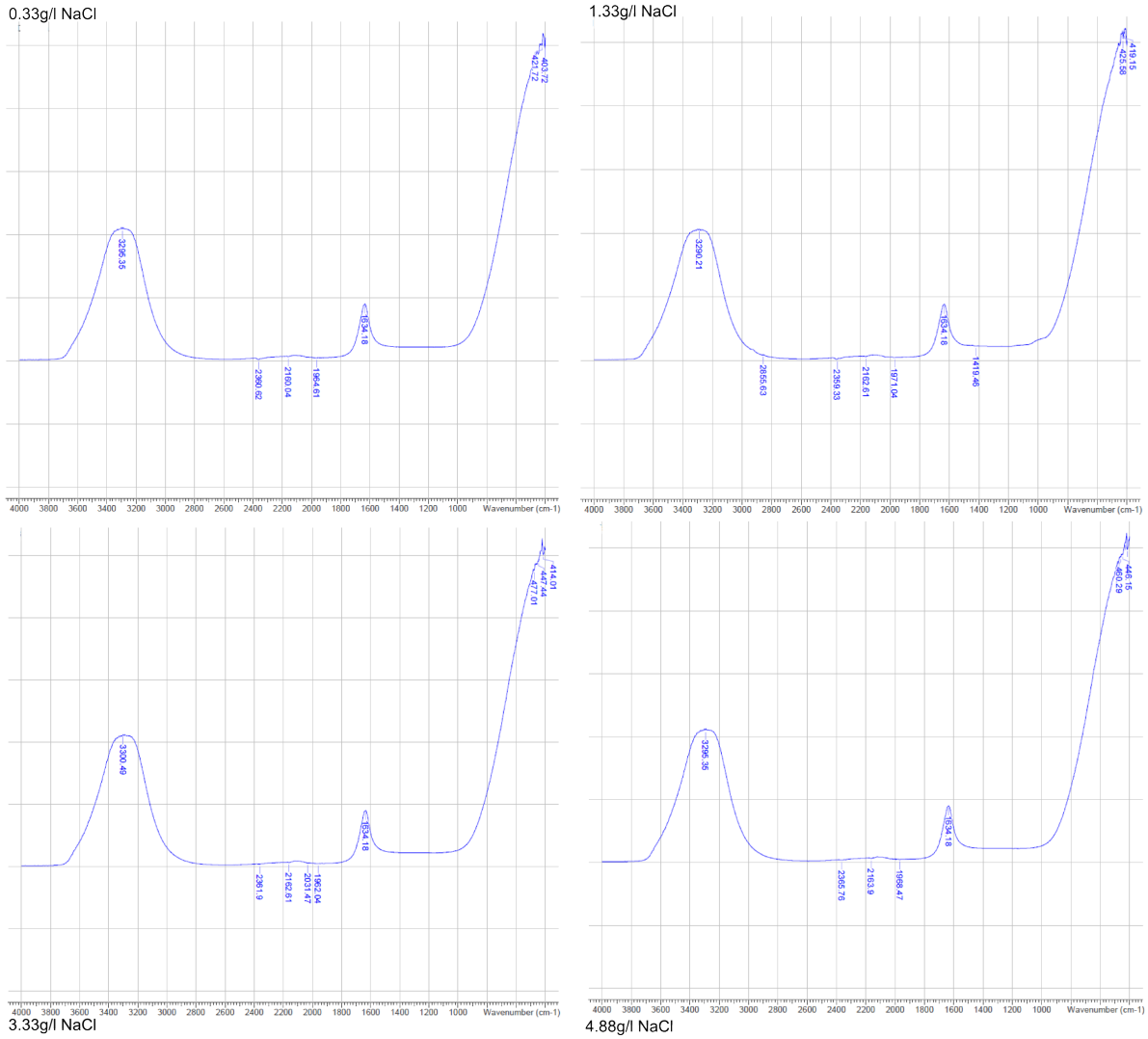

Figure 6: **FTIR spectra for populations in different salinity treatments**
Populations in the 0.33g/l treatment (A) showed a fingerprint region at 403.72cm^{-1} with intensity 3.479 and at 421.72cm^{-1} with intensity 3.497. Carbon-Carbon bonds were present with alkene (intensity 1.024 at 1964.61) and alkyne (intensity 1.032 at 2160.04cm^{-1}). CO_2 was present with intensity 1.01 at 2360.62cm^{-1}. Either OH or NH was present with intensity 2.044 at 3295.35cm^{-1}. Populations in the 1.33 g/l treatment (B) showed at fingerprint region at 419.15cm^{-1} with intensity 3.567 and at 425.58cm^{-1} with intensity 3.526. The population showed a peak of 1.113 for aromatics at 1419.46cm^{-1}. Carbon-Carbon bonds were present with alkene (intensity 1.022 at 1971.04cm^{-1}) and alkyne (intensity 1.031 at 2162.61cm^{-1}). CO_2 was present with intensity 1.008 at 2359.33cm^{-1}. Either OH or NH was present with intensity 2.032 at 3290.21cm^{-1}. Populations in the 3.33 g/l treatment (C) showed a fingerprint region at 447.44cm^{-1} with intensity 3.454 and at 477.01cm^{-1} with intensity 3.381. Carbon-Carbon bonds were present with alkene (intensity 1.024 at 1962.04cm^{-1}) and alkyne (intensity 1.033 at 2162.61cm^{-1}). CO_2 was present with intensity 1.019 at 2361.9cm^{-1}. Either OH or NH was present with intensity 2.061 at 3300.49cm^{-1}. Populations in the 4.88 g/l treatment (D) showed a fingerprint region at 446.15cm^{-1} with intensity 3.449 and at 447.44cm^{-1} with intensity 3.429. Carbon-Carbon bonds were present with alkene (intensity 1.023 at 1968.47cm^{-1}) and alkyne (intensity 1.031 at 2163.9cm^{-1}. CO_2 was present with intensity 1.018 at 2365.76cm^{-1}. Either OH or NH was present with intensity 2.053 at 3295.35. All treatments had an unidentified peak of intensity ~1.5 at 1634.18cm^{-1}.

treatment (g/l)	fingerprints (cm^{-1})	aromatics (intensity)	alkene (intensity)	alkyne (intensity)	CO_2 (intensity)	OH or NH (intensity)	C=O (intensity)
0.33	403.72 3.497		1.024	1.032	1.01	2.044	1.451
1.33	3.567 3.526	1.113	1.022	1.031	1.008	2.032	1.444
1.33	3.567 3.526	1.113	1.022	1.031	1.008	2.032	1.444
3.33	3.454 3.381		1.024	1.033	1.019	2.061	1.452
3.33	3.454 3.381		1.024	1.033	1.019	2.061	1.452
3.33	3.454 3.381		1.024	1.033	1.019	2.061	1.452
4.88	3.449 3.429		1.023	1.031	1.018	2.053	1.452
4.88	3.449 3.429		1.023	1.031	1.018	2.053	1.452

Table 1: **FTIR spectral intensities for populations from the 0.33g/l habitat in different salinity treatments**
Intensities of peaks varied amongst treatments with no clear trend for increase or decrease. Populations in 1.33g/l showed peaks for aromatic compounds not present in any other treatment.

Both phosphorous and amino acid quantities are increased in *D. magna* from the 3.33g/l habitat

FloatBarrier

amino acid	resonance in individuals from the 3.33g/l NaCl habitat (ppm)	resonance in individuals from the 0.33g/l NaCl habitat (ppm)
leucine	0.96	1
threonine	1.29	NA
alanine	1.48	1.48
methionine	2.14	2.14
lysine	3.24	3.27
glycine	3.57	3.57
phenylalanine	6.90	NA

Table 2: **Amino acids identified in proton NMR sprectre**
Seven amino acids could be assigned to spectra

Following Nagato et al. (2013), certain peaks in 2 are likely to be indicative of amino acids in *D. magna* with valine at 1ppm, leucine at 1ppm, isoleucine at 1ppm, threonine at 1.29pmm, alanine at 1.48ppm, arginine at 2ppm, methionine at 2.14ppm, glutamate at 2.3ppm, lysine at 3.24ppm, glycine at 3.57pmm, and phenylalanine at 6.9ppm. In the spectra obtained from the 0.33g/l culture, peaks were present at positions corresponding to leucine, valine, alanine, methionine, lysine, and glycine though all signals were below 0.1kHz. Signals of the 3.33g/l exceeded an intensity of $5x10_{-5}$ for all signals that would correspond to amino acids. However, the number of hydrogen atoms at the site of the peaks only 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.

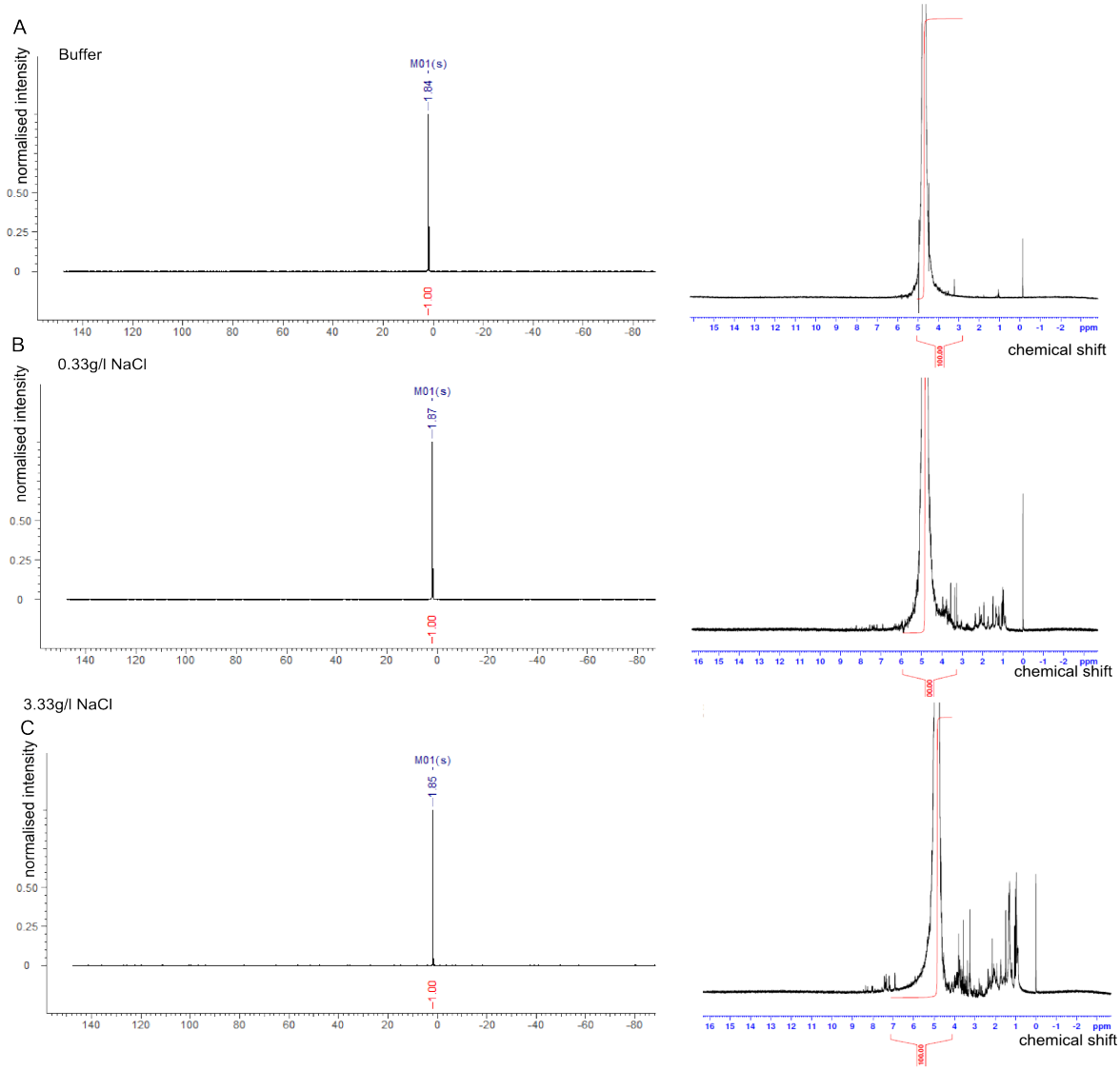

Figure 7: **NMR proton spectra and P31 spectre of *D. magna* grown in 0.33g/l (B) and 3.33g/l (C) alongside the buffer sprectrum (A)**
In the NMR proton spectra TMS is located at 0ppm and D_2O at 5.56-5.80ppm. NMR showed a normalised $P_3 1$ peak for *D.magna* grown in 0.33g/l at 1.86 and for *D. magna* grown at 3.33g/l at 1.85 with the buffer at 1.84 (see figure 8). Multiplets present in the proton spectrum were 2 hydrogens at 3.78ppm (intensity $>5x10^{-5}$), 3 hydrogens at 5.44ppm (intensity $1x10^{-4}$), 5 hydrogens at 0.98ppm (intensity $1x10^{-4}$), 6 hydrogens at 1.29ppm (intensity $1x10^{-4}$) and 7 hydrogens at 4.61ppm (intensity $1x10^{-4}$).

Discussion

West-Eberhard (1989) argues phenotypic plasticity to be an integral part of evolution in the light of the modern synthesis. As *D. magna* avoid, disperse, adapt or perish (Arnér & Koivisto 1993) in reaction to environmental heterogeneity, the fitness increase resulting from phenotypic plasticity causes a selection pressure. Polyphenysm in regard to pigmentation and body size of *D. magna* resulting from phenotypic plasticity has been shown here to occur alongside changes in population dynamics and further plasticity in behaviour, all in agreement with underlying metabolome changes. The plasticity occurs in both reaction to salinity stress and diet related stress. Such plasticity allows for populations of *D. mangna* showing the plastic response to display and increase in fitness.

Just below the reported LC50 (5g/l to 7g/) morphological changes occur in form of increased pigmentation for both populations and a thrifty phenotype (Hibshman et al. 2016) for the population from the

habitat with higher salinity. The phenotypic plasticity of the tail length is dependent on salinity, though not with the linear relationship suggested by Warren (1900). Pigmentation changes come from changes in carotenoid expression in *D. magna*. Increased caretenoid production has been associated with a stress response to UV exposure and diet which here suggests a harmful effect of algae accumulation as the populations showing increased pigmentation also have lower population sizes (De Meester & Beenaerts 1993).

While *D. magna* feed on algae, high concentrations as in the case of low population sizes have been shown to be harmful to the individuals. An excess of algae in the *D. magna* habitat causes eutrophication which impacts the swarms. It has been reported that *D. magna* are negatively affected by the algae they feed on (Bownik 2016). Such nutrient pollution halts population growth and has been shown here to cause behavioural anormalies that appear when the algae become attached to the *D. magna* individuals and imped their movement.

Survival appeared to be related to changes in the environment rather than to a specific salinity threshold as an increase of 1g/l NaCl caused population sizes of populations from both habitats to increase. As the population decreases for higher populations, this shows that the *D. magna* show parity variation in offspring number which suggest a higher reproduction rate for these populations. Such a higher reproduction rate is indicative of higher fitness. While offspring number increased as part of the plasticity response, drastic changes in salinity like from 0.33g/l NaCl to 4.88g/l NaCl caused populations that did not form swarms to crash. The changes in age structure confirmed studies showing that the maturation age of *D. magna* is highly plastic (Ebert 1994, Lampert 1993). Furthermore, the role of swarming becomes evident in that swarms have population dynamics different from smaller populations with higher survival for high salinities. On an individual level, salinity stress impairs avoidance of collisions and leads to swarms forming without swimming in the characteristic vortex structure. Individual behaviour and thereby the plastic response of each individual was dependent on the overall swarm and it's population dynamics. Helitrophism and vortex swarming were abandoned in high salinities, further adding to the plasticitiy of swarming behaviour. Salinity had an additional indirect effect on individual behaviour through population dynamics.

Biological interpretation of metabolome changes

Metabolome data extrapolated from FTIR spectra suggest a general slight increase in metabolism consistent with the costs of plasticity for the population from the 0.33g/l habitat. *D. magna* from the 0.33g/l habitat had their highest population sizes in 1.33g/l NaCl and aromatic compounds which are generally toxic to *D. magna* (Ikenaka et al. 2006) were found in *D. magna* from the 0.33g/l habitat exposed to the same salinity. Biotransformation of aromatic compounds involved cytochrome P450 (CYP). Such toxicity may be causal for the increase in population size for the populations and the resulting parity variation in offspring number, thereby confirming the higher reproductive rate seen in acute salinity stress (Khudr et al. 2018) is also present in chronic salinity stress. The increase of C=0 in combination with the peak that includes OH suggests an increase in acid production alongside the increase in offspring for the 1.33g/l population where both functional groups appear more frequently. An increase in acid production has been associated with many different metabolome changes, most commonly in fatty acids (Furuhagen et al. 2014). The increases in CO_2 across populations suggest oxidative stress as observed in Kim et al. (2010) and more to increase with salinity exposure.

Phosphorylation decreased as amino acid metabolism increased, suggesting an increase in energy usage. Such energy impairment was not indicative of 3.33g/l NaCl constituting a lethal level which suggests salinity to influence the *D. magna* metabolome differently from the way organophosphates were shown to in (Nagato et al. 2016). The population from the 3.33g/l habitat showed a higher metabolic rate in their habitat than the population from the 0.33g/l NaCl habitat did. Furthermore, the population from the 3.33g/l NaCl habitat had higher population sizes in high salinities. This suggests that the metabolomic stress of an original habitat with higher salinity enabled a plastic response to higher subsequent salinity exposure, thus preventing the populations from crashing. This is in agreement with the sublethal toxicant concentrations (here 3.33g/l NaCl in one of the habitats) causing a decrease in the *D. magna* net energy budget (De Coen & Janssen 2003).

The lack of signals indicating phenylalanine in the population from the 0.33g/l NaCl habitat suggests metabolome differences to manifest in hormonal differences, especially in regards to alterations in dopamine and octopamine as previously identified in (Nagato et al. 2013). Furthermore, the population from the 0.33g/l NaCl habitat lacks a signal for threonine present in the population from the 3.33g/l NaCl habitat which suggests the population to be under less stress as an increase of threonine has been asso-

ciated with salinity stress (Garreta-Lara et al. 2016). Garreta-Lara et al. (2016) identified the biosynthesis of secondary metabolites, central carbon metabolism in cancer, biosynthesis of amino acids, aminoacyl-tRNA biosynthesis, protein digestion and absorption, mineral absorption, carbon metabolism, arginine biosynthesis, oxocarboxylic acid metabolism, alanine, aspartate and glutamate metabolism, arginine and proline metabolism, and cysteine and methionine metabolism as metabolic processes affected in stressed *D. magna*. Combining the identification of single amino acids and the assumption that the the region at 4.61 is likely to include alanine, cystein, asparagine, and serine, the cysteine and methioniine metabolism which involves alanine, asparagine, and methionine is likely to be affected by the salinity stress.

Limitations

The analysis of the phenotypic and behavioural plasicity with the methods used here has a number of limitations. Data from a culture trained to survive in 2.88g/l NaCl was discarded as the stock culture was not stable enough to maintain the necessary population size for the behavioural assays. While the bioimaging allowed to measure compression of individuals as a behavioural trait it was not possible to measure it by observation which is why it has been excluded from the behavioural analysis. Futhermore, insufficient camera equipment did not allow for a mathematical evaluation of model predictions in comparison to actual behaviour as individual movement could not be tracked continuously.

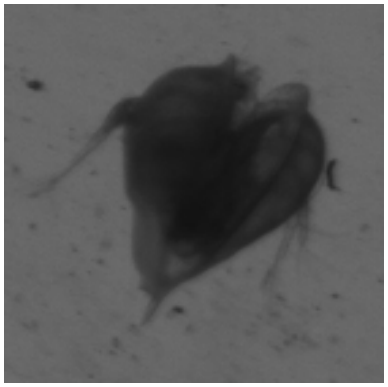

Figure 8: **Compression could also be behaviour affected by salinity and influencing other behavioural traits.**

The Mill at the University of Manchester is not a sterile environment and all sample preparations were undertaken in multi-user laboratories in both the Mill and Michael Smith building. Organisms the *D. magna* could have potentially been exposed to are *Anopholes spp.*, *Acyrthosiphon pisum*, *Blattodea spp.*, *Cupriavidus necator*, *Drosophila melanogaster*, *Escherichia coli*, *Haloferax mediterranei*. Contamination during the lyophilisation could explain the strong noise in the spectra FTIR spectra that could not be analysed. The $\mathrm{D_2O}$ used is over 10 years old which might have affected the NMR. Due to the NMR samples being whole organisms samples, spectra for nitrogen could not be taken, as the *D. magna* were not labelled with isotopes. Carbon spectra were disregarded due to noise. Furthermore, any signal noise produced by the *D. magna* endosymbionts could not be discarded due to the small sample size and the general noise in the spectra. The biological interpretation of the NMR data impeded by the lack of signal for glutamate and proline.

Future work

Metabolomics supplemented by a proteome analysis of the trained populations could provide further insights into the exact changes underlying the plastic response observed. Metabolome alterations that decrease the degradation of aromatic compounds leave sulfate as a product of the xenobiotic metabolism (Ikenaka et al. 2006). Measurements of internal sulfate concentrations would allow more detailed insights into the metabolic activity of *D. magna*. Resonances that previously could not be measured in the NMR due to noise in taken spectra, like nitrogen and carbon spectra, could be measured with a stronger NMR machine. Lipid measurements as in De Coen & Janssen (2003) may be performed using NMR as long as D_2O is replaced with chloroform as the resonance background. Though there are currently

no HPLC columns available for *D. magna* other proteomics methods including Bio-Rad protein assays as used in Pauwels et al. (2007) may be used targeting HSP60 and HSP70. Gene expression may be tested using bisulphide sequencing after methods used in Asselman, De Coninck, Beert, Janssen, Orsini, Pfrender, Decaestecker & De Schamphelaere (2016) will show further insights into whether the metabolome changes of serine and threonine were also associated with methylation of the amino acids. Transcriptome plasticity via alternative splicing has been suggested to have a significant effect on phenotypic plasticity (Mastrangelo et al. 2012). As the annotation of the *D. magna* genome improves, the effect of alternative splicing in the plastic response to salinity stress may be explored further. While Latta et al. (2012) proposed transcriptome plasticity in *D. magna*, the energetic costs of such plasticity are unclear as of now.Populations grown across further environmental heterogeneity including fluctuating habitats could show further deviations from the populations grown in 0.33g/l NaCl and 3.33g/l NaCl. A population trained in 2.88 g/l has been observed to fluctuate in population size by growing to large swarm before crashing. The changes in behaviour associated with this remain to be seen.

Conclusion

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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Appendix

Supplementary statistical data

variable	glm p-value	ANOVA F-value
exposure level	0.855503	0.8555661
individual		0.4208331
age = adult	0.310513	
age = juvenile	0.707961	
culture	0.000167	0.0001565 ***
no. swarm		0.5939399

Table 3: statistical results for the glm using the total population as a response variable

variable	LR χ^2	Df	Pr($>\chi^2$)
Swarm	25.265	7	0.000681 ***
initial_pop	12.104	7	0.097184 .
age	25.260	14	0.0320840 *
initial_sal	10.265	7	0.174070
sal_stress	53.570	28	0.002516 **

Table 5: There is no significant correlation between the tail length and the exposure level as previously stated by Warren (1900)

As the equipment necessary to film the Daphnia in a way that would allow to analyse the swarming using DaphTrack or ToxTrac which meant that the behaviour could not be compared to a adapted from Erdmann et al. (2004), Mach and Schweitzer (2007) and Ottermans et al. (2014). A *Daphnia magna* population growths with a rate of $[\mu] = d^{-1}$. It is determined using a life-table data on age-specific survival [l(a)]and age-specific fecundity [m(a)] $\sum m(a)l(a)e^{-\mu a}$ In their movement *Daphnia magna* can be regarded as active Brownian particles. Assuming that a single *D. magna* in medium is such a particle, its spartial position can be modelled by a vector $\vec{r}$. In their average movement they perform a 'hopping' motion with a speed of $4-16\frac{mm}{s}$ and a sinking rate of $3\frac{mm}{s}$ Their movement can be modelled as them being a single self-propelled active Brownian particle in a central field which is subject to noise.
Then the movement of the *D. magna* can be easily described by velocity dependent dissipation $\gamma(v)$.
For $\gamma(v) < 0$ energy is pumped into the *D. magna* and may be stored while for $\gamma(v) > 0$ the energy

uptake is insufficient. Following Rayleigh's law

$$\gamma(v) = \gamma_1 + \gamma_2 v^2$$

γ_1 and γ_2 are constants serving to declineate constant and velocity dependent contributions to the dissipation. The particle assumes a self-propelling velocity is

$$v_0^2 = \frac{\gamma_1}{\gamma_2}$$

which leads to the second law

$$\gamma(v) = \gamma_0(1 - \frac{v_0}{v})$$

For this law there is a singularity at $v \to 0$ while Rayleigh's law has a singularity at $v \gg v_0$.
If we first consider the motion of the *D. magna* on a two-dimensional plane, x-y, where they move with a velocity $\vec{v}$ with

$$\delta_t \vec{r} = \vec{v}$$

where $\vec{r} = \sqrt{x^2 + y^2}$ is their location on the plane we can use Langevin equations representing the balance of forces to describe the motion:

$$m\delta_t\vec{v} = -\gamma_0\vec{v} + de(t)\vec{v} - \Delta U(r) + \sqrt{2D}\vec{\xi}(t)$$

whereas the movement is described as

$$\frac{d}{dt}\vec{r} = \vec{v}; \frac{d}{dt}\vec{v} = -\gamma(\vec{v}^2)\vec{v} + de(t)\vec{v} - \Delta U(r) + \sqrt{2D}\vec{\xi}(t)$$

The relation between the energy depot and the non-linear friction function is given through

$$-\gamma(\vec{v}^2) = \gamma_0 - d_2 e(t) = \gamma_0 - \frac{d_2 q_0}{c + d_2(\vec{v}^2)}$$

with a zero for

$$\vec{v}_0^2 = \frac{q_0}{\gamma_0} - \frac{c}{d_2}$$

which means that active motion $|v_0|$ is possible for $q_0 > \frac{c}{d_2}$.
The included forces are the random force $\sqrt{2D}\vec{\xi}(t)$, where D is the strength of the noise; the confining restoring force $\Delta U(r)$ where $U(r)$ is the external potential energy, the passive friction $-\gamma_0\vec{v}$ and the force resulting from the conversion of energy out of an internal energy depot $e(t)$ into motion $de(t)\vec{v}$.
Returning to the noise $\vec{\xi}(t)$, for *D. magna* it includes the external sense that the *D. magna* get from the environment and therefore from the liquid medium. This could include thermal collisions, hydrodynamic fluctuations. Furthermore, the movement that the *D. magna* perform to not collide with each other can be considered.
The noise can be assumed to be a delta function of the form $\langle \xi_i(t)\xi_j(t) = 2D\delta_{ij}\delta(t-s)\rangle$ and is Gaussian distributed with zero mean.
For $m \equiv 1$ we can describe the time evolution of the stored energy $e(t)$ and thereby the feeding habit of the *D. magna* can be modelled indirectly.

$$\delta_t e(t) = q_0 - ce(t) - dv^2 e(t)$$

q_0 is the energy available from the *D. magna's* energy uptake, $ce(t)$ is the energy deducted for metabolic requirement and $dv^2 e(t)$ the energy deducted for the *D. magna's* movement. c and d are constants. q_0, the available energy flow represents the food availability.
Because the energy uptake is slow in comparison to the dissipation, it can be eliminated so that $de(t) = 0$ and therefore

$$\gamma(v) = \gamma_0 - \frac{q_0}{c + dv^2}$$

where γ_0 is the dissipation constant in the limit of high particle velocity.
The self-propelling velocity of the *Daphnia* at zero effective dissipation is

$$v_0^2 = \frac{q_0}{\gamma_0} - \frac{c}{d}$$

To solve the Langevin equation for the balance of forces, using the third dissipation law but bifurcation the bifurcation parameter is

$$\mu = \frac{q_0 d}{c\gamma_0}$$

For $\mu < 1$ the motion of the *D. magna* is random around the symmetry point of the central potential while for $\mu > 1$ the motion is a pair of noisy limit circles.
Since *D. magna* is heavily dependent on phototaxis, the attraction to light becomes significant in describing the organism's movement. It can be determined using the environmental potential of the organism

$$\Delta U(\vec{r}) = \omega_0^2\vec{r} = \frac{a}{2}\vec{r}^2$$

This makes motion with a frequency of ω_0^2 on a closed x-y plane possible. The movement is then according to the self-propelling velocity $|\vec{v} > 0|$ on limit circles. There are also trajectories in the x-y plane. However, we cannot assume that the individuals move identically. Some of the individuals will be juveniles and therefore have different rates for energy conversion. This leads to a modification where the strength of the power dissipated in motion becomes noisy

$$d \to d_0 + v(t)$$

in which $\nu(t)$ is the noise. Since it is an internal fluctuation it is variable within the population. This is the population noise d, Gaussian distributed and delta correlated with standard deviation ω_d. It leads to a noisy dissipation

$$\gamma(\nu) = \gamma_0 - \frac{q_0}{c+[d_0+\nu(t)]v^2}$$

Using this in combination with

$$m\delta_t\vec{v} = -\gamma_0\vec{v} + [d_0 + \nu(t)]e(t)\vec{v} - \Delta U(r) + \sqrt{2D}\vec{\xi}(t)$$

it is possible to obtain trajectories and measure directly correlating with experimental data. For some of the organisms the energy conversion is not efficient enough to reach the limit cycle and therefore they participate in Brownian motion around the fix point.
Due to their phototaxis, *D. magna* motion tends to be around a central attractant. Therefore, it can be said that their cycling is not an emergent or self-organised inherent property of their swarm.
High density swarms are even capable of vortex motions. The vortex is a collective motion with local interactions. This is a result of a symmetry breaking. However, within a swarm the interactions between the organisms as single ABPs have to be considered. Within the vortex, the *D. magna* swim in the same direction for high population densities.
At small swarm density, the motion is mainly in the horizontal plane. The circling can therefore be simplified to a two-dimensional model. For the amount of circling, the probability distribution $P(\theta)$ of the heading angle, θ, can be measured. The motion shows twin peaks since the D. magna appear to swim into two directions at equal probability. The probability distribution $P(r)$ of the distance r that a *D. magna* would have to the light shows twin peaks suggesting the same for the angular momentum as for the probability distribution of the heading angle. The angular momentum distribution $rho(L)$ has L for $m = 1$ described as

$$L = r \times \nu$$

Since the *D. magna* swim in a swarm, no matter how many they are the many particle active Brownian particle theory is a more precise representation of the swarm dynamics. The attractive interaction summed over the entire swam leads to the initial Langrevin equation representing the balance of forces on one of the *D. magna*, i, in the ensemble

$$m\delta_t\vec{v}_1 = -\gamma_0\vec{v}_i + [d_0 + \nu(t)]e_i(t)\vec{v}_i - \kappa_h[\vec{r}_i - \frac{1}{N}\sum_j \vec{r}_j] + \sqrt{2D}\vec{\xi}(t)]$$

where κ_h is the coupling constant in the term that replaced the force generated by the external potential. This suggests that the internal swarm interactions have a stronger effect that internal noise.
The energy uptake of the i^{th} particle is

$$\delta_t e_i(t) = q_0 - ce_i(t) - [d_0 + \nu(t)]\vec{v}_i^2 e_i(t)$$

The consideration of the noise $\nu(t)$ results in a Gaussian distribution of d_i.
In many cases and independent external noise $\vec{xi}_i(t)$ will apply to the individuals.
In their swarming the individuals will initially be randomly distributed but then form the previously mentioned patterns.
To avoid collision the *D. magna* will have short range repulsion.
Regarding an Euclidian distance between to agents $r_{ij} = \|r_{ij}\| = |r_i - r_j|$ the repulsion between to individuals can be regarded as

$$V_1(r_{ij}) = \frac{c}{(r_i j)^n} n \in \mathbb{R}$$

where c is a constant.
If we conclude that i differs from j and that is a prerequisite of the model, we can apply the Elementary Theory of Order (Kittel 2005). For N ABPs the entropy becomes

$$S = 2Nk_B[(1+P)ln(1+P)ln(1-P)]$$

The number of arrangements G is

$$G = [\frac{N!}{[\frac{1}{2}(1+P)N]![\frac{1}{2}(1-P)N]!}]^2$$

Therefore and following the free energy $F = E - TS$ the equilibrium order is when said energy is a minimum with respect to the order parameter P, leading to

$$4NPU + Nk_BT \ln \frac{1+P}{1-P} = 0$$

The short–range parameter r is a measure of the fraction of the average number q of nearby ABPs that are different. The total possibility of such arrangements is eight and therefore

$$r = \frac{1}{4}(q-4)$$

As *D. magna* live in a low Reynolds number environment the laminar flows of water mediate the interaction between individuals. Considering dipole like flow around a moving *D. magna*, j, the flow field of j at distance r_ij at the location of particle i a net velocity v_F is present at location i. The hydrodynamics are then defined by

$$\vec{F} = \kappa_F \vec{v}_F$$

Which, using the Oseen law, turns the balance of forces into

$$m\delta_t \vec{v}_i = -\gamma_0 \vec{v}_i + \kappa_F \vec{v}_F - \kappa_h[\vec{r}_i - \frac{1}{N}\sum j\vec{r}_j] + \sqrt{2D}\vec{\xi}(t)$$

where $\kappa_F \vec{v}_F$ is generated by all particles. Therefore, the flow field becomes

$$\vec{v}_F(\vec{r}_j) = \sum_{j \neq i} \frac{R}{r_ij}[\delta + \frac{\vec{r}_{ij} \otimes \vec{r}_{ij}}{r_{ij}^2}]$$

valid for $r_{ij} \gg R$

where R is an effective hydrodynamic radius of these particles and $\otimes$ is a tensor product.
An alternative writing would be

$$\vec{v}_F(\vec{r}_j) = \sum_{j \neq i} [\frac{R}{r_{ij}}\vec{v}_j + \frac{\vec{r}_{ij} \otimes \vec{r}_{ij}}{r_{ij}^2}]$$

valid for $r_{ij} \gg R$

The final consequence of the hydrodynamics is that the entire swarm will rotate in one direction in case of swimming to closely.
The swarm size N can be related to the phase transition with which they swim by

$$F_{L+}(N) = |\bar{x}_{L+} - \bar{x}_L| = |2x - 1|$$

with

$$\bar{x}_{L+} = \frac{1}{s_m N} \sum_{k=1}^{s} \sum_{n=0}^{m} N_{L+}^{(k)}(t + n\Delta t$$

where $N_{L+}^{(k)}$ is the number of agents found with a positive angular momentum.