Response of *Daphnia magna* from Natural Populations to Ultraviolet Radiation

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Master Thesis

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Abstract

Diel vertical migration (DVM) is performed by various zooplankton taxa. The general movement pattern of DVM has been well studied while the possible clonal differences of this movement largely remain unknown. My master thesis focus on investigating the effects of environmental stressors on *Daphnia magna* strains isolated from natural populations. Here I maintained 11 strains of *Daphnia magna* originating from natural populations from lakes and ponds in southern Sweden. Their response under three consecutive treatments of UV radiation (UV turned off; UV turned on; UV turned off) was observed and the swimming speed and depth were analyzed. Results showed that *D. magna* from natural populations may respond to UV radiation with increasing swimming speed and depth. After UV exposure the swimming behavior remained similar to the phase when UV radiation exists. Difference in swimming behavior among strains under neutral environmental conditions was found. Furthermore, this difference disappeared under UV threat and all strains then performed a similar pattern of movement.

Keywords: *Daphnia magna*, swimming behavior, natural population, environmental stressors, UV radiation

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1 Introduction

1.1 Backgrounds

Animal movement is common and diverse. Different species of animals can for example fly, glide, run, or swim. When responding to various environments, they respond with different forms of movement as behavior. Therefore, movements are of great importance in ecosystems (Nathan et al, 2008). Zooplankton is heterotrophic plankton that drifts and swims in oceans, seas and freshwater bodies (Thurman & Burton, 1997). They perform diel vertical movements (DVM) to feed on phytoplankton and escape environmental threats such as predators or UV damages. DVM is performed in both marine and freshwater environments, with a migration depth up to 100 meters (Lampert, 1989). Copepods, for instance, can swim up to the surface at dusk and retreat back to deeper depths at dawn (Yoshida et al, 2004). This phenomenon suggests that changes in light intensity during the day is one of the main factors influencing DVM (Ringelberg, 1995).

Like other zooplankton taxa, *Daphnia magna* also performs DVM (Lampert, 1989). *D. magna* can swim to deeper waters to avoid being harmed by UV radiation (Hansson et al in 2016), and *D. magna* can perform vertical migrations repeatedly in response to fluctuating UV radiation, e.g. variations in cloudiness.

D. magna are small-sized cladocerans and usually have a body lenght of 2 to 5 mm. *D. magna* is usually the dominant plankton keystone species in the food webs of many lakes and ponds. They are filter feeders, feeding on suspended particles in water such as *Scenedesmus* and *Chlamydomonas* (Ebert, 2005). Planktivorous fishes are predominant predators of *D. magna* (Lauridson, 1996). With all these features, *D. magna* has been a popular model species in the domain of aquatic ecology (Jaromir, 2011).

The life cycle of *D. magna* is composed of a dominant asexual reproductive phase also known as cyclic parthenogenesis and possibly a short sexual reproductive phase (Fig 1) (Ebert, 2005). Kleiven et al (1992) reported that the induction of sexual reproduction of *D. magna* requires multiple factors, including shorter daylight, food limitation and chemically mediated crowding (chemical cue produced by *D. magna* of high density in water). Thus, when the environmental conditions are favorable for rapid population growth, *D. magna* would prefer to reproduce asexually and form a clonal population. Harsh environments, on the contrary, may cause the female *D. magna* to produce a son and diapausing ephippial eggs intended for sexual reproduction (Ślusarczyk et al, 1999). Sex ratio of *D. magna* is significantly affected by environmental conditions (Hobaek et al, 1990). Hence under certain environmental conditions, parthenogenetic reproduction generates females with the same genotypes of the mother (Eads et al, 2007) maintaining clonal strains of *D. magna*.

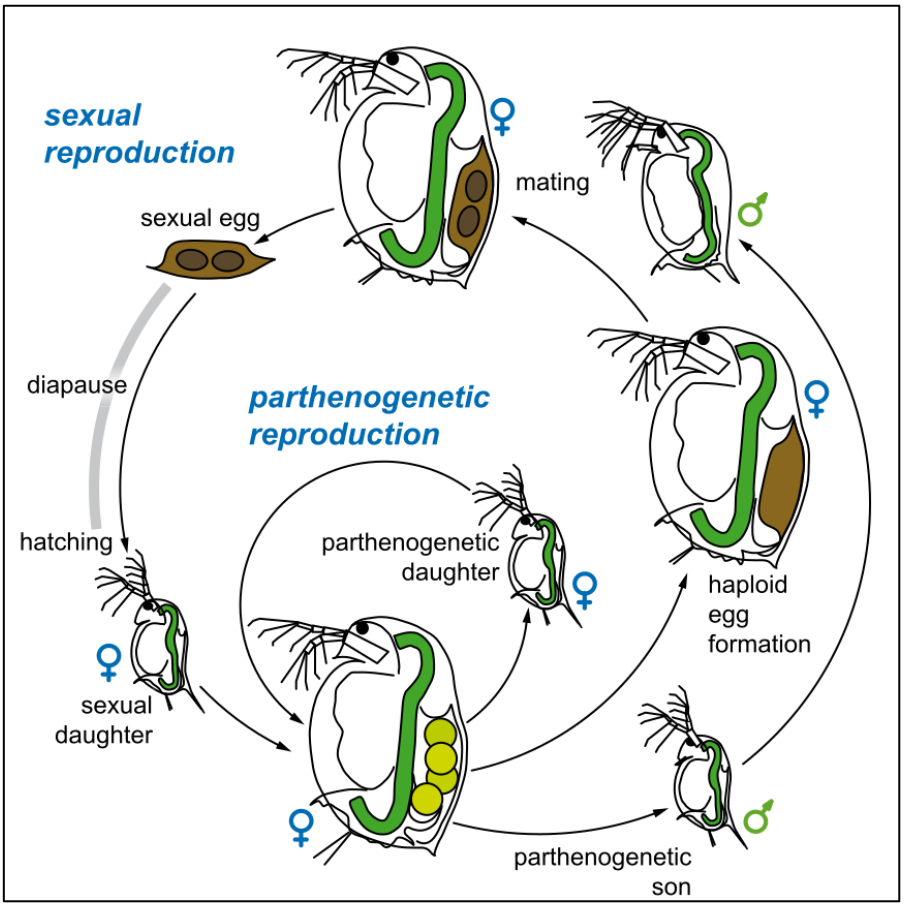

Fig 1. Life Cycle of *Daphnia magna*. *D. magna* performs both sexual and parthenogenetic reproduction responding to different environmental conditions. Image downloaded from https://upload.wikimedia.org/wikipedia/commons/3/37/DaphniaMagna_LifeCycle_DVizoso.svg.

Many studies have discussed the possible triggers or impacts of ultraviolet radiation on *D. magna*. Yet part of them only discussed certain behavioral parameters affected by UV radiation instead of tracking the dynamic process of movement. Huebner et al in 2009 stated that UVB radiation would significantly affect the offspring generation of *D. magna*. After exposed to artificial UVB radiation, *D. magna* would produce offspring generation with significantly lower production and survival. Other researches focused more on parameters of biochemical or toxicological aspects instead of swimming behavior of *D. magna*. For example, the toxicity of sulfonamide was reported to be enhanced under natural sunlight with the factor of UVA light (Jung et al, 2007).

Few studies have compared the specific swimming behavior with and without the treatment of UV radiation. Heuschele et al in 2017 conducted an experiment using nanoparticles to investigate the induced DVM of *D. magna* with UV radiation turned off, on and off consecutively. Their results of water depth over three UV phases provided evidence of

consistency in swimming velocity of different *D. magna* individuals through two generations when UV radiation was absent. The consistency of swimming behavior discovered in this study was determined at the level of individuals. Application of such pattern of swimming behavior of *D. magna* in a higher level of strains remained unknown. Thus, difference in swimming behavior induced by UV radiation through different strains from the same population would be a worthwhile topic to study. As a newly developed technique for tracking movement of small-sized organisms, usage of nanoparticles had great importance in assessing dynamic swimming behavior of zooplankton taxa.

1.2 Aims and hypotheses

During my MSc thesis, I evaluated the impact of ultraviolet radiation as an environmental stressor on *D. magna* swimming behavior (swimming speed and swimming depth) originated from natural populations using nanotechnologies. The aims of my MSc project were to investigate the impact of UV radiation as an environmental stressor on *Daphnia magna* isolated from natural populations (Bysjön in Southern Sweden) using swimming behavior defined as speed and depth as well as standard deviations and fitness through generations. I hypothesized 1) that *D. magna* from natural populations would perform DVM pattern movement when exposed to short-term UV threat. 2) Moreover, I hypothesized that under neutral conditions, strains from the same location may perform swimming behaviors that are different from each other since genetic difference may be the main factor. 3) When UV radiation exists, swimming behavior of different strains was expected to be similar due to plasticity caused by environmental threat would cover the factor of strain difference.

2 Materials and methods

2.1 *Daphnia magna* strain collection and maintenance

Samples were collected from lake Bysjön located in southern Sweden (see Appendices Table A1). *Daphnia magna* clones originating from Lake Bysjön (55.67424, 13.54653) were maintained in a laboratory of Lund University. The following procedures of collection and isolation were intended for samples collected directly from the field. The subsequent maintenance procedure was the same for samples from field and laboratory.

Samples were collected using a zooplankton net with a mesh with a pore size of 200 µm. Environmental parameters for each sampling sites were measured and reported in Appendices (Table A1). Zooplankton samples were stored overnight at 4°C before isolation. During isolation, the taxonomy and gender of *D. magna* were investigated under a light microscope (Olympus SZX7, Japan, x10) and compared to the taxonomy reference Fauna Sinica. Individuals were isolated from the field sample using a pasteur plastic pipet (612-4536, VWR International, Belgium) and observed under the microscope.

Selected female *Daphnia magna* were transferred individually to 60 mL filtered copper-free water using a vacuum filtration device (514-0332, VWR International, USA) in a 100 mL glass bottle. The bottles were maintained under light:dark cycle of 14:10 hours of 50 µmol photons.m^{-2}.s^{-1} at 17-20 °C. The rest of Daphnia specimens were discarded.

Strains of *Daphnia magna* were fed twice per week, with 1 mL of pure culture containing approximately 10^5 cells of *Scenedesmus sp*. Daphnia cultures were regularly gently shaken to resuspend the algae that sank to the bottom of the bottle.

All strains of *Daphnia manga* were maintained up to 3 parthenogenic-generations to exclude maternal effects before performing behavioral tests. At each generation, daughters were separated from the mother and dispatched individually in new bottles.

2.2 Swimming Behavior

Investigation of the swimming behavior of *Daphnia* was performed in the NanoBiology Lab (NBL) following a protocol adapted from Ekvall et al (2013). The protocol was subdivided into five consecutive steps.

2.2.1 Step 1 - Experiment set-up and acclimation period

The basic set-up of movement recording included a cubic aquarium (20*20*85 cm) with dark and transparent glass, 8 lens over the top of aquarium and 4 cameras on the side of aquarium (Fig 3). The lens on the top could create LED light and UV radiation at the same time. Cameras on the side could record the movement of organisms in the aquarium from 4 different angles. A computer was connected to the cameras as the control panel of the experiment.

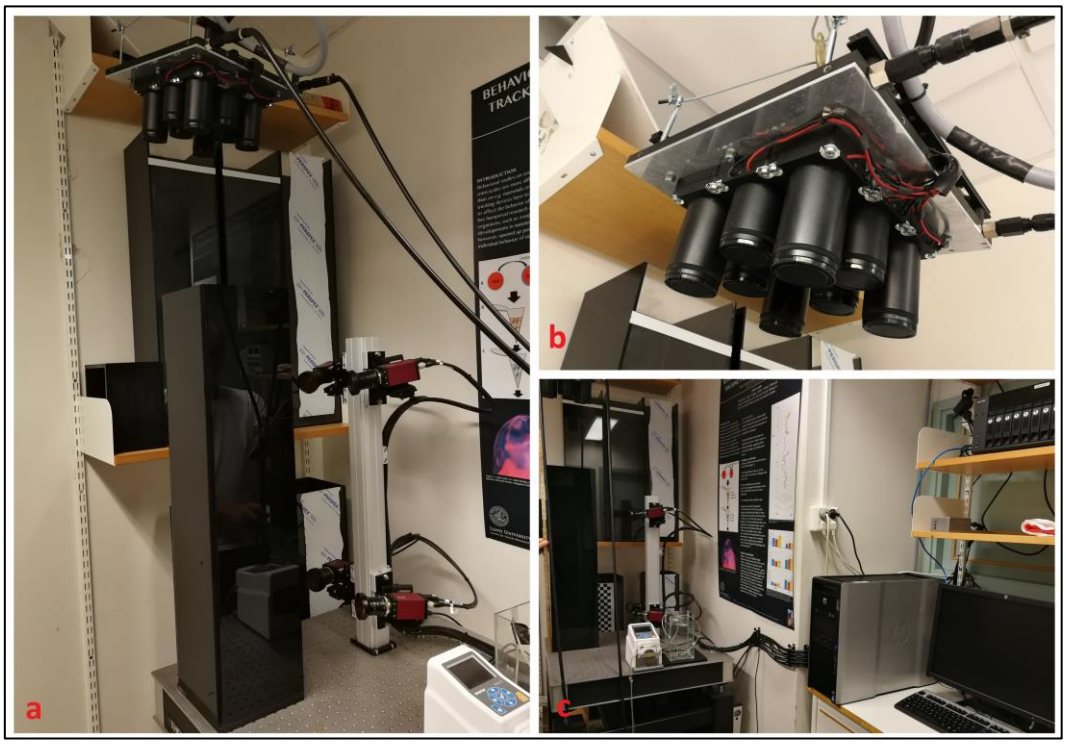

Fig 3. Set-up of recording: a) The 30 L plastiglas lidless aquarium with 8 lens on top and 4 cameras on the side. Every plane except the one facing cameras is dark in order to restrain UV radiation inside the aquarium. b) Elevatable 8 lens over the top of the aquarium to produce UV radiation directing downwards. c) Computer connected with lens and cameras as the control panel. (Photo by Luo Zheng)

Before tracking the swimming behavior, a preincubation period was applied to each strain of *D. magna*. Each Individual was transferred into a 100 mL glass jar with approximately 100 ml of copper-free water from the aquarium. The jar was incubated for about 12 hours under blue LED lights (470 nm) for the *D. magna* to adapt the light. This procedure aimed at acclimatizing each individual to the aquarium environment in which they were recorded.

2.2.2 Step 2 - Labeling individual

The labeling of individuals was performed in two times: a Quantum dot (Qdot) solution was first prepared and then the individuals were labeled.

To prepare the Qdot solution, a volume of 50 µL of 8 mM 585 yellow ITK Carboxyl Quantum dot (Life Technologies) was added to 400 µL 10mM borate buffer, pH 7.4 and mixed well. Then 4 mg Polylysine dissolved in 80 µL 10 mM borate buffer, pH 7.4 was added to the Qdot solution and mixed well. Subsequently, 46 µL of 10mg/mL N-ethyl-N'-dimethylaminopropyl-carbodiimide (EDC) was added to the Qdot/Polylysine solution and mixed well. The tubes were incubated in the dark (microtubes covered with aluminum foil) at room temperature for approximately 1 hour 20 minutes to have Qdot solution. All samples were mixed several times during the incubation.

Individuals *Daphnia magna* were transferred separately into 2 mL microtubes. The water was removed from the microtubes up to cover the organism. A volume of 250 µL copper-free water was added to the microtube. Later 8 to 10 µL (depend on the size of the individual) of the polylysine conjugated Qdot solution was added to the tube and mixed gently using the pipette. Tubes were incubated again in the dark for 1 hour. After incubation, the excess of Qdot conjugate solution was removed and the organism was washed with 1 mL of copper-free water gently for at least three times.

Labeled individuals were transferred into the aquarium (Fig 3) containing 30 L of copper-free water using a plastic pipet. During the transfer, bodies of individuals should not enter in contact with air to avoid forming air bubbles which would enhance buoyancy causing individuals to float.

2.2.3 Step 3 - Recording

During the tracking period, the lab remained in the dark, without ambient interference of light. Recording of the labeled Daphnia individuals using the software STEVICORD (ver 14.07.15b) for 3 min. The recording was initiated when an individual reached the upper 25 cm of the aquarium. An automatic recording programme was started to record for 1 min without UV radiation, then turn on UV radiation (150 mW/cm^2) for 1 min, and ended with 1 min with UV turned off. The whole video file contained 3 min with 6 FPS.
The above procedure of recording was repeated for 3 times as soon as the Daphnia swam back to 25 cm depth. If Daphnia stayed at the bottom for over 30 min, it was placed back in culture for another tracking experiment. *Daphnia magna* were picked out from the aquarium after tracking and their image captured under a light microscope using the software Infinity Capture.

2.2.4 Step 4 – Tracking (Triangulation)

After recording, video files generated from STEVICORD were first analyzed by a Matlab based programme Daphnia Tracking v1.7 (based on Matlab R2017a) to generate 4 tracks, one per camera. Redundant noise spots and reflection of tracks were removed using Matlab-based software DaphniaTracking V1.7. The 4 cleaned tracks were merged into a generated 3D

tracking files. The tracking files were triangulated for the analyses of real-time swimming speed and depth.

2.2.5 Step 5 – Data analysis

Mean maximum and minimum swimming speed and depth, as well as the standard deviation of *Daphnia magna* individuals were estimated using R software (R x64 3.2.5). Then the data were analyzed with SPSS (IBM SPSS Statistics 25). The analyses were performed for all strains and generations within and among locations.

3 Results

A total number of 67 individuals of 11 strains from Bysjön were recorded with UV treatment and tracked to extract swimming behavior data. Through statistical analyses in R studio, summarized data of each individual were presented in Appendices (see Table A2). The data of swimming behavior include mean, maximum, minimum and standard deviation values of swimming speed and depth during the tracking in arena. Due to high mortality in certain generations of some strains, 15 individuals of generation G3 in 4 strains (B36, B39, B42, and B44) were also recorded and tracked as reserve samples. With 67 individuals and 3 replicates, the total number of observations was supposed to be 20. Due to mortality and slow movements of *D.magna* during experiment, 27 of them did not have 3 replicates due to mortality and slow movements and the total number of observation was 167.

For all individuals tested, mean swimming speed and depth of *D. magna* before, during and after UV treatment was presented in Fig 4 and Fig 5. The total mean speed before, during and after UV treatment were 17.5, 20,5 and 20.2 mm/s. Analysis of one-way ANOVA indicated that swimming speed of *D. magna* increased significantly during and after UV treatment ($F_{2,498)}$= 16.288, $p < 0.001$ for global test; mean speed before vs UV: $p < 0.001$; before vs after: $p < 0.001$, Tukey's test). Since the observation of each individual was divided into 3 stages, the total sample size was 1 less than 3 times 167, which was 500 in the one-way ANOVA test. This also applied to the analyses between mean depth and UV treatments. After the UV treatment, swimming speed of *D. magna* showed no significant changes in the last 1 min of recording (mean speed UV vs after: $p > 0.05$, Tukey's test). Same pattern occurred in the mean swimming depth over three stages of experiments that mean depth significantly increased during and after the UV treatment ($F_{2,498} = 271.578$, $p < 0.001$ for global test; mean depth before vs UV: $p < 0.001$, before vs after: $p < 0.001$) while the depth of before, during and after UV treatment were 146, 481 and 471 mm. No significant difference was found between the on-going and after UV treatment stages either (mean depth UV vs after: $p > 0.05$, Tukey's test).

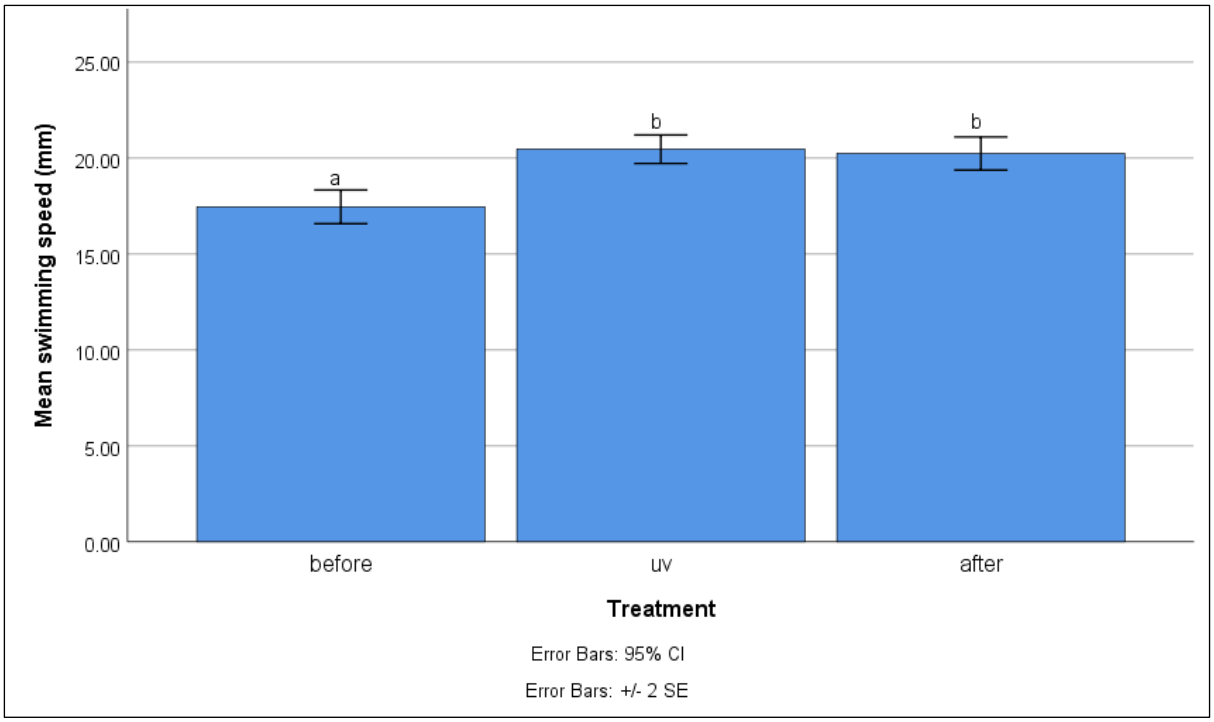

Fig 4. Differences of mean swimming speed of *D. magna* between different stages of treatment (before UV treatment, during UV treatment, after UV treatment). Different letters above bars (a, b) indicate significant differences between groups (n=167).

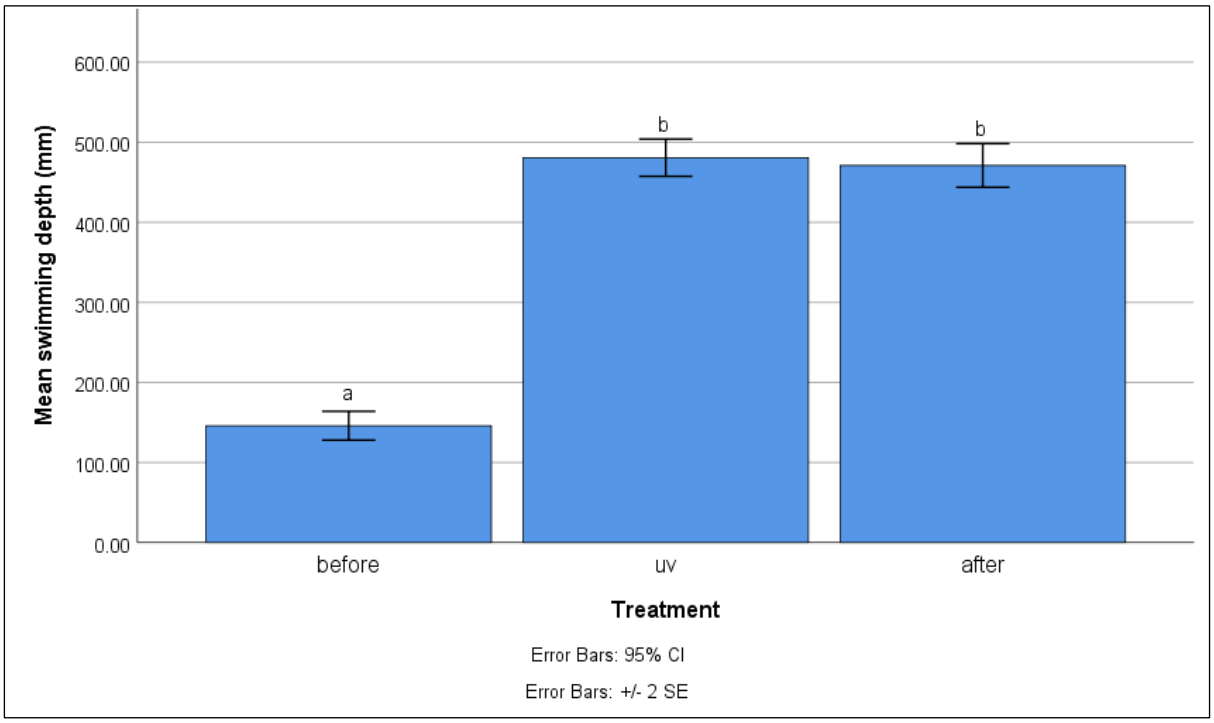

Fig 5. Differences of mean swimming depth of *D. magna* between different stages of treatment (before UV treatment, during UV treatment, after UV treatment). Different letters above bars (a, b) indicate significant differences between groups.

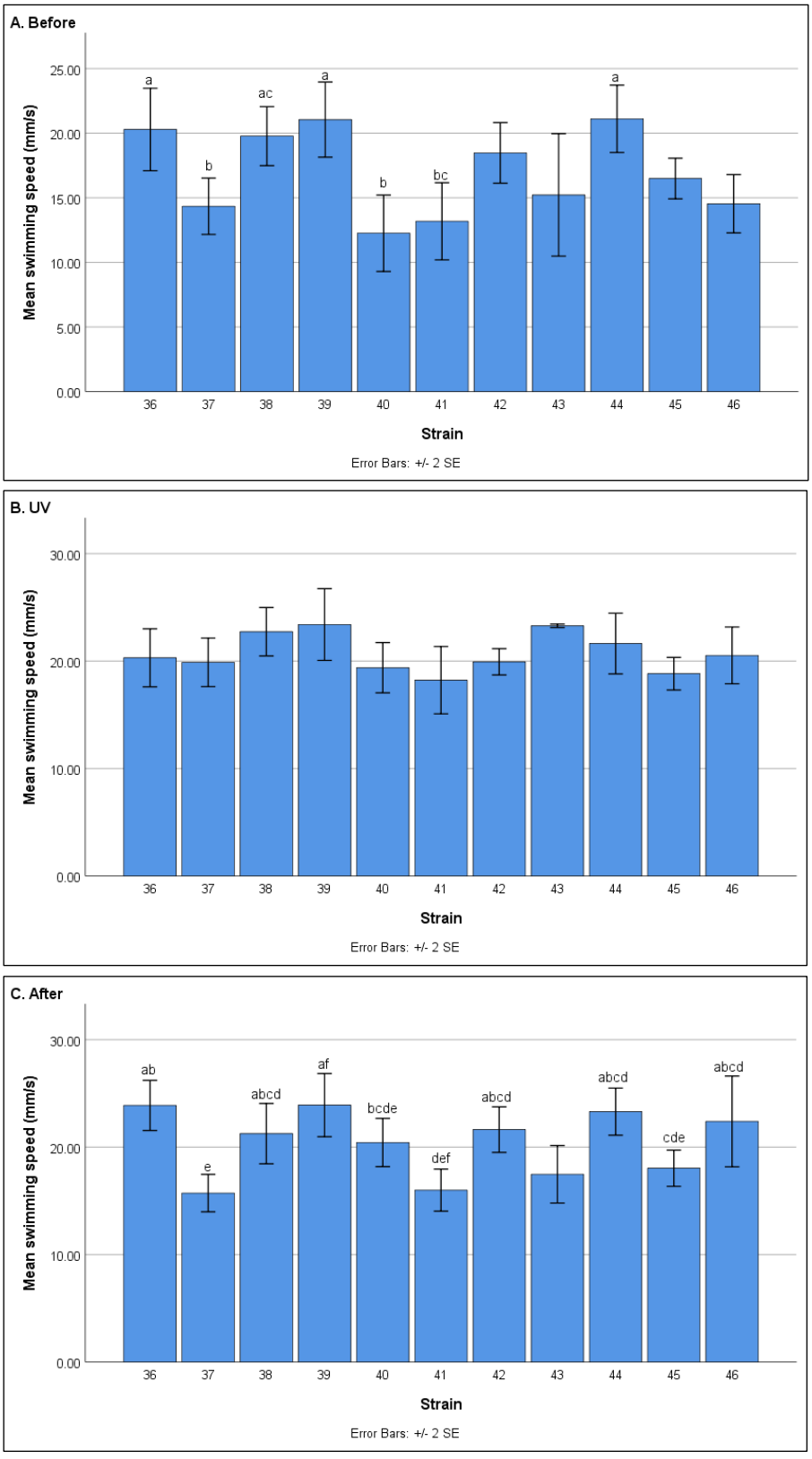

Fig 6. Differences of mean swimming speed of *D. magna* between different strain groups (36 = strain B36, 37 = strain B37, et cetera) of three stages (before UV treatment; during UV treatment; after UV treatment). Bars marked with completely different letters suggested significant difference between groups. Bars with no letters suggested no significant

differences between groups. For each strain (36, 37, 38…46), n=16, 23, 19, 15, 7, 9, 26, 3, 10, 31, 8.

For the first minute of recording before UV treatment, significant differences were found between 11 strains of *D. magna* (Fig 6 A. $F_{10,156} = 4.870$, $p < 0.001$ for global test). Among all the strains, 6 strains in 10 pairs through pairwise comparisons were significantly different from each other (strain 36 vs 37: p = 0.020; strain 36 vs 40: p = 0.028; strain 36 vs 41: p = 0.041; strain 37 vs 38: p = 0.031; strain 37 vs 39: p = 0.005; strain 37 vs 44: p = 0.025; strain 38 vs 40: p = 0.042; strain 39 vs 40: p = 0.011; strain 39 vs 41: p = 0.015; strain 40 vs 44: p = 0.024; strain 41 vs 44: p = 0.036, Tukey's test). For the second minute of recording when UV was turned on, one-way ANOVA showed significant difference in speed between 11 strains (Fig 6 B. $F_{10,156} = 1.919$, $p = 0.046$ for global test; The total number of observation was 167 as mentioned above). Yet no significant difference was observed in the corresponding pairwise comparisons where all pairs had a p value > 0.05 in Tukey's test. The final minute of recording after UV treatment showed significant differences in swimming speed between 11 strains of *D. magna* again (Fig 6 C. $F_{10,156} = 5.951$, $p < 0.001$ for global test). Within the strains, 10 strains in 10 pairs through pairwise comparisons were significantly different from each other (strain 36 vs 37: p = 0.000; strain 36 vs 41: p = 0.007; strain 36 vs 45: p = 0.007; strain 37 vs 38: p = 0.015; strain 37 vs 39: p = 0.000; strain 37 vs 42: p = 0.002; strain 37 vs 44: p = 0.003; strain 37 vs 46: p = 0.042; strain 39 vs 40: p = 0.008; strain 39 vs 45: p = 0.009, Tukey's test). In general, swimming speed of *D. magna* differed significantly through different strains in the beginning stage of treatments before UV was on. During the UV treatment, swimming speed of all strains turned to be similar without significant difference and after UV was off, speed through strains became significantly different again.

One-way ANOVA test suggested significant difference in swimming depth between 11 strains of *D. magna* before UV treatment (Fig 7 A. F (10,156) = 4.870, p = 0.043 for global test). Within the strains, no significant difference between any 2 strains was found through pairwise comparisons. During UV treatment, depth of strain 44 was significantly deeper than strain 37 (Fig 7 B. $p < 0.05$) and were the only pair of strains with significant difference in this stage ($F_{10,156} = 3.308$, $p = 0.001$ for global test). The depths of different strains in the following stage after UV treatment were not significantly different within the groups ($F_{10,156} = 1.236$, $p > 0.05$ for global test).

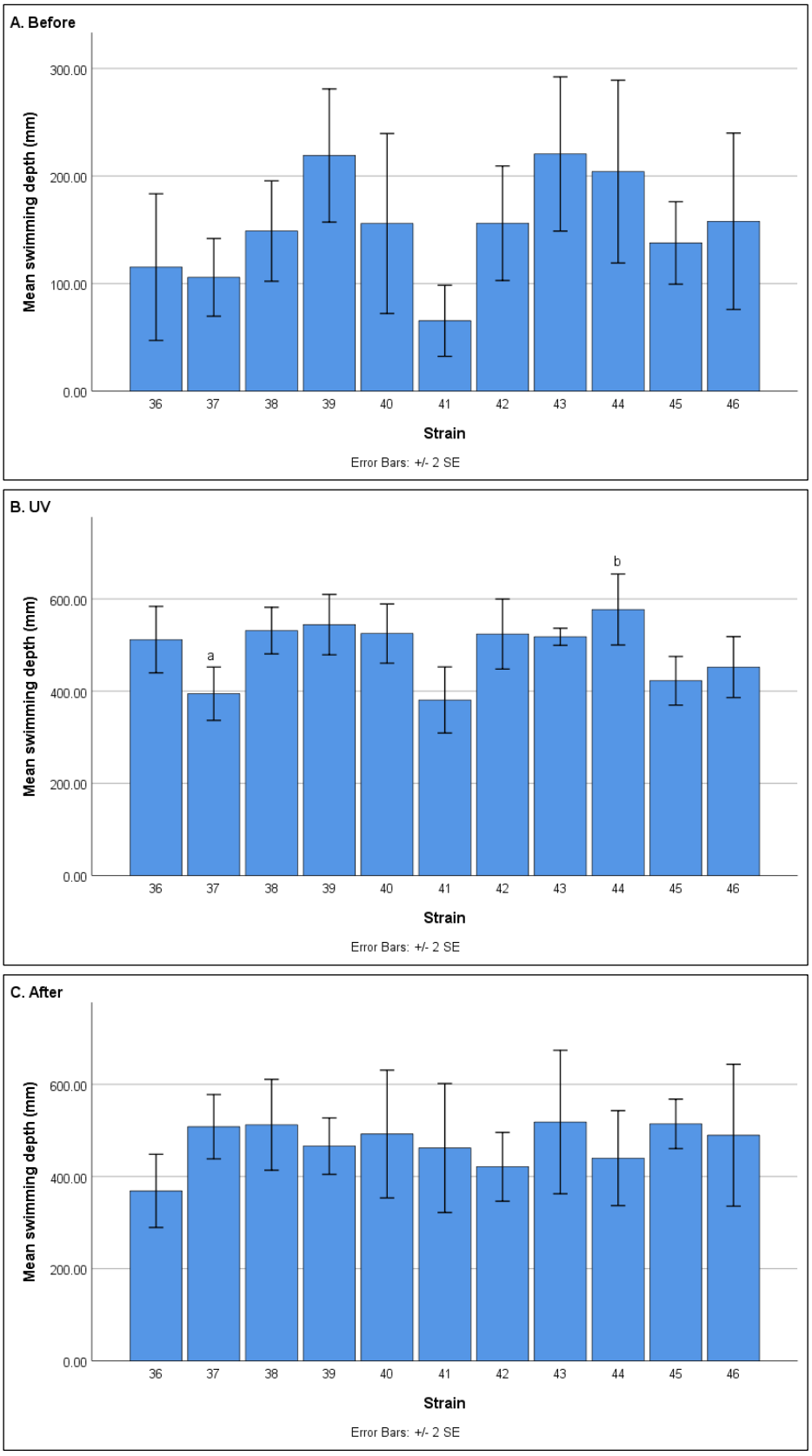

Fig 7. Differences of mean depth of *D. magna* between different strains (36 = strain B36, 37 = strain B37, et cetera) during three stages (before UV treatment; during UV treatment; after UV treatment). For each strain (36, 37, 38…46), n=16, 23, 19, 15, 7, 9, 26, 3, 10, 31, 8. Bars

marked with completely different letters suggested significant difference between groups. Bars with no letters suggested no significant differences between groups.

4 Discussion

4.1 General swimming behavior in response to UV radiation of *D. magna*

Results from Fig 4 and Fig 5 indicate avoidance behavior by *D. magna* when exposed to environmental stress, such as UV radiation. Mean swimming speed of all strains increased significantly from 17.45 mm/s to 20.46 mm/s when UV radiation exist, while mean swimming depth increased significantly from 145.86 mm to 480.75 mm. This demonstrates that when detecting stressors in shallow depth, daphnids would not only perform vertical movement by swimming downwards, but also swim with significantly faster speed to escape from potential threats. Research of Rhode et al in 2001 stated that UV in sunlight could be an additional factor triggering DVM of genus *Daphnia*, suggesting when performing DVM under natural environments, *D. magna* may also accelerate instead of swimming constantly into a deeper layer.

The three-stage recording of this experiment was also intended to determine the swimming behavior of *D. magna* when UV radiation disappeared. Insignificant differences in speed and depth between, during and after UV radiation suggest that *D. magna* may keep the similar swimming behavior at least for a short period when environmental stressor no longer exist. This was different from my hypothesis that their swimming behavior would change back to be similar to before UV treatment. One possible explanation could be that the 1 minute time period in the experiment was insufficient for *D. magna* to acclimate the change of stressor. In spite of the behavior of moving back to upper depth caused by DVM, this "back and forth" pattern of swimming may not apply to *D. magna* in a short time scale of minutes. With a longer time of acclimation after radiation, *D. magna* may swim back to the depth near the surface. Another possible reason could be the negative effect of nanoparticles applied to *D. magna* as labeling technique. During the recording procedure, some individuals were observed to stay near the bottom of the aquarium after UV radiation with slow locomotion. It has been reported that inner cores of nanoparticles quantum dots contain cadmium and selenium that in spite of protective coatings of nanocrystals, may still occur at levels above toxic threshold levels of *Ceriodaphnia dubia* through transfer (Bouldin et al, 2008). Lovern and Klaper reported in 2006 that exposure to fullerene (C_{60}) nanoparticles would negatively impact *D. magna* and cause certain mortality. It is also possible that the behavior of some *D. magna* individuals was affected by long incubation time when treated with Qdot solutions during experiments. On the other hand, research of Lard et al in 2010 reported insignificant difference in the behavior, reproduction, or survival between *Daphnia* treated with quantum dots and those not treated. Besides, the handling procedure of transferring *D. magna*

individual into aquarium using pipets may cause negative effect on the mobility of individuals. Inappropriate operation in this process may cause actual damage to *D. magna* such as dropping them to aquarium above water surface instead of letting them swim out with the tip of pipet beneath surface.

4.2 Analyses of swimming behavior over strains of *D. magna*

Mean swimming speed during the UV treatment and depth before UV treatment of *D. magna* showed no significant difference through different strains in Fig 6 B and Fig 7 B using Tuckey's test, with the fact that one-way ANOVA showed significant difference for global test ($p < 0.05$). This complexity of data analysis could be caused by statistical rather than experimental factors. There could be several possible reasons why post-hoc test indicated insignificant difference while the global effect was shown to be significant. For one hand, the outcomes of global effect could be weakly significant results. In statistics, this situation may occur when p value of ANOVA is equal or close to the significant level, which is 0.05 in the present research. The p values of ANOVA test were 0.046 for swimming speed over strains during UV radiation and 0.043 for swimming depth over strains before UV radiation. Both values were over 0.04 and were near 0.05, hence the significant global effect could be a possibly weak result. Technically, the significant level of 0.05 was a manually selected standard of over 95% accuracy to reject null hypotheses instead of a concrete conclusion. Therefore, the actual insignificant difference of pairwise comparisons was still acceptable when p values were near 0.05. The high number of factor levels in this paper could also cause this conflicting results. There were 11 strains as factors when comparing mean speed and depth and the total number of pairs between groups in the analysis were 55 in this case. This comparatively high number of pairwise comparisons may result in a more penalized p value, reducing the risk of rejecting null hypotheses. Conservative feature of post-hoc test may be another factor. Generally, a more conservative multiple comparison test would be more likely to reject significant difference. All post-hoc tests in this paper were Tukey's test, which is a comparatively conservative test allowing one to make all combination of pairwise comparisons.

It is possible to choose another test rather than Tukey's test to acquire statistically significant differences in mean speed during UV and depth before UV, yet this may cause a loss in precision of the analyses. Besides, Tukey's test provided convincing analyses on the mean speed before and after UV as well as depth during and after UV. The three stages of treatment were divided parts from a consistent recording data, hence it would be more precise and convincing to use a coherent post-hoc test for all three groups. On this basis, it would be proper to draw the conclusion that both mean speed during UV radiation and depth before UV radiation of *D. magna* had insignificant difference over strains according to Tukey's test.

4.3 Effects of UV radiation on swimming behavior of *D. magna* over strains

With the assumption of data analyses mentioned above, results of mean swimming speed over strains suggested that under natural condition, *D. magna* from the same natural population of different strains would present different swimming behavior (see Fig 6 A). Dodson et al in 1997 also demonstrated that the overall pattern of *Daphnia* reactions to food and light levels were unique for different clones. Although *Daphnia* didn't perform a classic pattern of DVM that swim upward again after radiation threat, empirical evidence was provided in this experiment that different behavior among strains occurs.

When UV radiation as environmental stressor exists, *D. magna* of different strains would perform vertical movement in similar swimming velocity despite their differences under normal status before UV. Therefore evidence was provided to prove the hypothesis that *D. magna* from natural populations would have plasticity to some degree that when certain threats occur, they would react in a similar swimming pattern. Due to possible ensuing impacts of environmental threats, *D. magna* kept the similar swimming speed after UV radiation as presented in Fig 6 C. This also suggested that *D. magna* from different strains might have similar acclimation behavior after UV was turned off. In general, the inducible response of environmental stressors on *D. magna* could be a bigger factor than differences in strains, at least for the respect of swimming speed.

Results of mean swimming depth over strains indicated that in most cases, *D. magna* of different strains may migrate to similar depth when UV radiation was detected (Fig 7). In Fig 7 B, *D. magna* of strain 44 distributed significantly deeper than those of strain 37 when UV was turned on. This pair was the only significantly different group among all strains. This difference in depth could be caused by the different sample sizes of the two strains. A total number of 10 individuals from strain 37 were tested while only 3 from strain 44 were tested due to mortality in culturing individuals. An alternative explanation was that the strains difference in swimming depth occurs, while similar swimming depth of other strains was the result of plasticity from the same natural population. Besides, tolerance or adaptation to UV radiation could be different among strains. Oda et al in 2007 reported difference among strains in sensitivity of *D. magna* insect growth regulator, fenoxycarb. It is possible that there is difference among *D.amgna* strains in swimming depth when facing environmental stress which may be covered by plasticity in population. Based on insignificant results of other strains, *D. magna* may stay at similar water depth under natural status or UV stressors. The maximum depth in the experiment was 750 mm determined by the size of the aquarium. Considering the depth of sampling site Bysjön (1190 mm, see Table 1), the results might be somewhat different if the experiment was conducted with a deeper aquarium. The depth of most strains showed no significant difference before, during and after UV radiation, thus supported the idea that *D. magna* from natural population would tend to respond in similar swimming behavior when exposed to environmental stress.

The study of Heuschele et al in 2017 implied that swimming behavior of *Daphnia* could be partially explained by individual behavioral types and state-dependent decisions instead of a general movement pattern that could apply to the whole population. This individual difference of distribution in water bodies could be a possible factor behind the difference through strains illustrated in the present thesis. While different individual movement types exist, difference among individuals could be enlarged when they are originated from independent strains. Study of Heuschele also provided evidence that this consistency in behavior would break down when UV radiation was on, which also supported the finding in my thesis that behavior of Daphnia tend to be similar when environmental stressor exist. Daphnia in Heuschele's study swam back to shallow depth after exposure to UV while individuals in my experiment were more likely to stay at deeper depth. The main reason for this difference in distribution after UV treatment could be the different time of phases that each phase in Heuschele's experiment last 3 min. In the present study, the 1 min phase resulted in a movement different from general DVM that *D. magna* prefers to stay at the bottom after 1 min exposure to UV radiation. A longer acclimation time of 3 to 5 min, for instance, may induce the classic pattern of movement that when threat disappears, individuals swim upwards again.

From a larger perspective, the strain movement types could be applied to other zooplankton that performs DVM movement or other behavior that different strains behave differently while they share similar general patterns. Changes of environmental stressors are larger factors when affecting the behavior of zooplankton taxa, that the different strain type behavior would break down and show convergence in some degree when threats occur.

Several aspects in this research were worthwhile to be expanded as proper directions for future studies. The intensity of UV radiation in this study was fixed. Different levels or intervals of UV radiation may trigger different results. Difference among strains may emerge even when threat exists when UV radiation was at low level and not strong enough to affect their movement types. Analyses among individuals within an independent strain may reveal the relationship between individual-based behavior and strain-based behavior. Another inspiring field of study is the comparison between offspring individuals of parents treated with or without UV radiation, or other stressors such as predator cues.

5 Conclusions

Under neutral circumstances, *D. magna* from natural population would emerge difference through strains in swimming behavior. When facing environmental stressors, swimming speed and depth of *D. magna* would increase significantly with similar movement pattern that the difference through strains disappeared.

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8 Glossary

Nanotechnology:
The technique using nanoparticles to label small-size organisms in order to track their movement under certain condition.

Population:
Ecological explanation: A population corresponds to the organisms of the same species, which live in a particular geographical area, and have the capability of interbreeding. In this research, all *Daphnia magna* living in the same lake is a geographical population.
Genetic explanation: All organisms of the same species sharing the same genotype according to different markers.

Quantum dot:
Quantum dots are small particles of only several nanometers in size. It exists many types of quantum dots. They can emit light of specific frequencies when excited by a source of light. In this research quantum dot was excited by Blue Light and allow the labeling of *Daphnia magna*.

Offspring:
Asexual: Young born of living organisms produced by a single organism without mating.
Sexual: Yong born of living organisms produced by two mating organisms.

9 Appendices

Location	Code	Type	Coordinator (N, E)		N	Predators	Water Temperature (°C)	Depth (cm)	pH	Conductivity (µS/cm)
Sydvatten	P	Pond	55.660709	13.541140	28	Insects, worms, mayfly, dragonfly nymph	14.1	9.5	9.4	281
Norra Fäladen	N	Pond	55.728175	13.222649	1	Insects, Chaoborus, Hydra	13.4	50	8.4	662
Bysjön	B	Lake	55.674240	13.546529	11	NA	14	119	9.0	328

Table A1. Sample information and physical parameters of sampling locations. All samples in TableSample were collected on May 9th 2017. N = Number of strains. NA = Not available

location	strain	individual	generation	mean speed (mm/s)	max speed (mm/s)	min speed (mm/s)	sd speed (mm/s)	mean depth (mm)	max depth (mm)	min depth (mm)	sd depth (mm)
B	36	A	0	15.21	42.76	0.41	7.2	275.38	642.31	12.48	216.14
B	36	AA	1	22.72	57.28	0.57	8.91	345.23	762.79	11.86	246.33
B	36	AAAa	3	26.54	70.35	2.65	10.92	226.51	763.55	9.87	270.74
B	36	AAAb	3	25.38	61.54	1.09	9.19	475.78	764.12	234.38	164.92
B	36	AAAc	3	18.61	148.97	0	15.29	392.77	761.88	9.61	300.83
B	36	AAAd	3	23.13	61.37	1.73	11.05	453.26	763.12	122.89	216.47
B	36	AAAe	3	23.43	71.16	0.5	11.7	260.74	762.73	11.51	261.15
B	37	B	0	20.84	48.76	2.47	8.23	389.23	763.83	10.98	263.68
B	37	BA	1	15.73	49.61	0	6.27	495.86	762.07	231.7	192.95
B	37	BAB	2	16.98	59.88	0.61	8.95	283.49	660.73	12.4	240.15
B	37	BAC	2	12.97	52.65	0.7	7.19	227.37	494.89	14.12	163.74
B	37	BB	1	13.31	34.54	1.37	5.91	326.23	644.58	18.07	247.09
B	37	BBA	2	16.3	70.23	0	9.6	424.37	762.05	122.23	197.09
B	37	BBE	2	10.88	47.47	1.72	5.31	265.48	438.17	146.43	77.32
B	37	BC	1	21.19	56.73	1.75	8.24	363.77	762.79	15.97	296.55
B	37	BCE	2	17.36	48.72	1.22	9.24	303.18	762.85	18.51	256.13
B	38	B	0	25.73	63.92	2.8	9.57	372	763.47	17.82	238.7
B	38	BA	1	17.85	52.32	2.06	8.27	388.24	715.66	49.17	256.05
B	38	BAE	2	26.49	74.37	0	12.03	491.36	764.54	76.3	242.28
B	38	BBA	2	24.09	63.71	1.28	11.4	330.18	763.53	27.25	238.44
B	38	BBB	2	13.78	40.97	0	9.32	319.76	764.71	16.05	332.53
B	38	BBC	2	23.99	76.62	2.57	10.26	428.6	762.55	18.88	265.38
B	38	BC	1	19.46	52.22	1.93	7.3	437.99	762.5	97.05	232.3
B	38	BD	1	17.85	52.32	2.06	8.27	388.24	715.66	49.17	256.05
B	39	D	0	26.46	245.45	0.28	16.42	344.91	761.24	9.38	262.49
B	39	DB	1	26.23	83.13	2.93	9.42	435.83	764.87	128.37	203.21

location	strain	individual	generation	mean speed (mm/s)	max speed (mm/s)	min speed (mm/s)	sd speed (mm/s)	mean depth (mm)	max depth (mm)	min depth (mm)	sd depth (mm)
B	39	DBDa	3	24.28	171.8	0.33	13.18	400.74	764.39	56.35	220.98
B	39	DBDb	3	21.18	65.14	2.61	9.77	383.1	763.97	53.04	211.27
B	39	DBE	2	25.53	347.89	0	19.82	427.38	763.43	172.93	201.04
B	39	DBF	2	15.93	45.55	2.16	6.12	406.42	663.54	18.2	196.08
B	40	B	0	19.71	60.9	2.98	8.78	502.18	763.16	140.08	253.11
B	40	BAA	2	16.65	122.9	1.84	9.4	429.61	762.97	238.4	182.78
B	40	BC	1	16.09	58.24	0.69	8.91	280.03	729.7	15.92	249.12
B	41	A	0	19.05	42.91	1.85	7.25	408.54	763.02	53.82	266.58
B	41	AA	1	13.4	45.43	1.14	6.44	211.09	480.46	15.5	150.38
B	41	AAB	2	14.49	63.96	2.55	7.19	304.99	762.46	14.66	271.92
B	42	CB	1	23.43	58.82	1.44	9.04	309.38	612.83	16.7	194.5
B	42	CBA	2	25.09	67.36	1.35	10.41	322.98	764.22	13.05	276.17
B	42	CBAa	3	18.96	87.98	0.54	11.23	441.75	763.38	57.88	276.16
B	42	CBAb	3	26.7	65.8	2.03	7.87	476.33	762.36	182.36	192.61
B	42	CBAc	3	15.98	41.58	2.11	5.81	455	761.91	129.13	198.7
B	42	CBAe	3	18.5	48.61	2.05	7.34	367.27	761.95	85.18	230.03
B	42	CBB	2	16.86	46.96	1.28	7.61	354.29	762.6	14.6	269.06
B	42	CBBa	3	14.59	277.57	1.04	14.72	386.52	763.04	10.76	326.53
B	42	CBBc	3	20.86	48.08	1.8	6.82	190.81	450.47	70.64	100.9
B	42	CBBe	3	16.9	39.2	1.89	5.52	376.4	763.03	58.46	240.51
B	43	B	0	18.9	68.94	2.09	8.98	449	764.68	215.57	189.46
B	43	BBA	2	16.99	375.16	0.11	15.4	325.17	764.02	119.72	182.47
B	44	A	0	24.02	58.16	2.2	8.62	388.6	763.32	10.12	247.34
B	44	AA	1	22.84	54.86	3.13	7.97	464.57	763.7	108.49	221.64
B	44	AB	1	23.28	57.22	2.29	8.52	349.2	762.26	44.62	242.98
B	44	ABA	3	17.4	51.34	2	7.06	425.83	763.67	137.71	198.01
B	45	A	0	16	49.23	1.19	6.37	305.91	685.88	12.42	247.95
B	45	AB	1	23.84	65.83	2.62	8.25	395.93	695.5	95.43	216.74
B	45	ABB	2	16.71	60.8	1.36	8.18	433.42	763.16	63.17	269.54

location	strain	individual	generation	mean speed (mm/s)	max speed (mm/s)	min speed (mm/s)	sd speed (mm/s)	mean depth (mm)	max depth (mm)	min depth (mm)	sd depth (mm)
B	45	AC	1	21.42	65.21	3.73	6.75	439.53	730.15	162.65	187.77
B	45	ACB	2	18.88	42.61	1	6.66	336.03	763.09	32.37	245.54
B	45	ACF	2	19.11	56.47	1.67	6.26	425.73	760.03	92.79	210.62
B	45	ACH	2	12.72	136.13	0.88	8.52	244.94	502.57	18.71	184.19
B	45	AD	1	13.34	38.12	2.6	4.74	285.24	447.38	110.62	118.02
B	45	ADE	2	13.27	33.5	3.9	4.31	286.43	382.77	156.61	66.77
B	45	AE	1	20.5	64.18	1.3	8.58	288.56	658.98	16.25	227.52
B	45	AEA	2	18.82	56.83	1.35	6.96	433.95	760.11	113.95	237.06
B	46	D	0	20.17	67.48	0.92	9.98	313.67	606.83	39.93	201.11
B	46	DA	1	25.43	56.82	3.22	12.05	347.03	760.58	33.11	202.46
B	46	DAA	2	15.37	66.61	1.16	6.81	447.97	644.28	298.34	114.05
B	46	DAB	2	19.25	102.41	0.89	8.39	423.6	763.34	18.68	287.96

Table A2. Swimming behavior of *D. magna*. sd = standard deviation. B = Bysjön