Kentucky Journal of Undergraduate Scholarship

Volume 4 | Issue 1 Article 2

2020

Adaptive Tolerance to Sodium Chloride in Daphnia magna

Brady P. Parlato
Georgetown College, bradyp@georgetowncollege.edu

Rick Kopp
Georgetown College, Rick_Kopp@georgetowncollege.edu

Adaptive Tolerance to Sodium Chloride in Daphnia magna

Cover Page Footnote

The authors thank Dr. Jana Henson for maintaining the lab space for this study in good condition, and for clarifying how to refer to commercially-purchased materials used in this study. We also thank Mr. Cameron Kenner for feeding the study organisms for a brief period when the authors were unable to do so. Finally, we thank Mrs. Autumn Pittman and Dr. Timothy Griffith for providing input on the statistical tests used in this study.

Adaptive Tolerance to Sodium Chloride in *Daphnia magna*

Brady P. Parlato & Rick Kopp
Georgetown College

Abstract: *Salinity levels of some freshwater systems have been altered by humans, posing a threat to aquatic life. We hypothesized that a Daphnia magna population pre-treated with a low concentration of NaCl would develop greater salinity tolerance than an untreated population. Two Daphnia magna cultures with 60 individuals each were established, one started with 1.00 ppt NaCl and increased by 0.20 ppt weekly, and the other with pure spring water. After 4 weeks, 40 neonates from each culture were individually placed in a solution of 2.30 ppt NaCl (separately determined to be the LC_{50}) for 48 hours. Survival was significantly greater for the experimental group ($LR = 7.5$, $df = 1$, $p < 0.05$) and significantly exceeded the expected 50% survival rate ($\chi^2 = 6.4$, $df = 1$, $p < 0.05$). This finding suggests that Daphnia populations can evolve or acclimatize to increase their survival in high salinity habitats.*

Keywords: *Daphnia, water fleas; salt runoff; salinity tolerance; microevolution; acclimatization*

Toxicants in freshwater systems are known to harm native organisms. For example, when contaminated water from mining operations in Romania spilled into several rivers throughout Eastern Europe in 2000, tens of thousands of freshwater fish were killed. Antal et al. (2013) found that it took almost ten years for the population of the affected fish species to increase and the water quality to significantly improve. Likewise, Farombi et al. (2007) found elevated levels of Zn, Cu, Cd, As, and Pb in the kidneys, livers, and hearts of catfish in the Ogun River in Nigeria. Based on the proximity of the study area to six major industries in southern Nigeria, the researchers attributed the elevated heavy metal concentrations in these catfish to industrial pollution. While numerous studies have investigated the negative effects of aquatic toxicants on target organisms like fish, Coldsnow et al. (2016) noted that less attention has been paid to the effects of these toxins on zooplankton taxa, such as Cladocerans in their natural environments.

Water fleas (Order Cladocera) are small crustaceans mainly found in freshwater habitats. According to Forró et al. (2007), there are about 620 known species of Cladocerans, although some researchers estimate that the real number is even higher. As reported by Pennak (1989), their bodies (0.2 to 3.0 mm), covered by a protective carapace, typically include compact heads, two fanlike antennae used in locomotion, and thoracic legs used in filter-feeding. According to DeMott (1982), members of the genus *Daphnia* feed on a wide variety of different microorganisms, ranging from bacteria, to protists of the genus *Paramecium*, and to algae such as *Scenedesmus*. *Daphnia* reproduce predominantly through parthenogenesis, where the young are able to develop from the mitotic division of unfertilized eggs. As discussed by Ebert (2005), adult females produce a clutch of parthenogenetic eggs every 4 days, which have an average gestation period of 3 days. On average, 8 offspring are produced by each female per reproductive cycle, and offspring can reach sexual maturity within 6 days. Furthermore, Pennak (1989) described how females will only lay parthenogenetic male eggs and sexual female eggs during periods of environmental stress, such as a decrease in water temperature in the winter. These darker, fertilized eggs, called ephippia, are more durable, and this sexual reproduction helps to promote genetic diversity within the population.

Larger species of *Daphnia*, like *Daphnia pulex* and *Daphnia magna,* are often viewed as keystone species due to their trophic roles. Indeed, Dodson (1972) reported that *Daphnia* can serve as the main food source for many planktivorous organisms, such as *Chaoborus* larvae. In addition to trophic roles, a Swedish study by Persson et al. (2007) has suggested that *Daphnia* also serve as keystone grazers by helping to provide algae-derived energy to higher order animal consumers in lake communities. Likewise, Cottingham and Schindler (2000) discussed how *Daphnia* have been known to dominate the zooplankton communities of fishless temperate ponds. However, researchers such as Steiner (2004) have also shown that water temperature and pH play key roles in regulating the biomass and thus trophic roles of larger *Daphnia* species in temperate zooplankton communities.

In addition, larger *Daphnia* species are generally good subjects to use in toxicity tests due to the ease and low cost of raising them in a laboratory setting. For example, to avoid the high cost of physical and chemical methods for evaluating environmental toxins, Tonkopii and Iofina (2007) used *Daphnia magna* to determine the presence of anthropogenic pollutants in freshwater systems in Japan. One study has even suggested that *Daphnia* may be able to serve as a more ethical and economical replacement for mammals when pre-screening for the toxicity of certain chemical agents. Guilhermino et al. (2000) found that *Daphnia magna*, while not as sensitive to certain toxic compounds as rats, still had toxicity test results with a high degree of validity for 15 tested compounds.

Some scholars have suggested that *Daphnia* may develop an increased tolerance to certain toxicants in their aquatic environments. There are several different means by which *Daphnia* and related zooplankton are hypothesized to develop increased salt tolerance. For example, Ortells et al. (2005) suggested that *Daphnia* come to tolerate high salinities by engaging in sexual reproduction and producing more durable, ephippia eggs. However, Bailey et al. (2004) found that higher salinity levels significantly increased *Daphnia* mortality in ephippia collected from sediments of the Great Lakes in North America. Thus, the more likely explanation for *Daphnia* salinity tolerance is gradual adaptation in response to high salinity water that develops over multiple generations. For example, Liu and Steiner (2017) found that clonal lines that were raised over several generations from populations taken from high-salinity ponds (based on conductivity readings) showed greater survivorship in acute toxicity tests than those developed from low-salinity ponds. However, they also found that *Daphnia* from higher salinities were generally smaller in size, suggesting that increased salt tolerance may come with evolutionary tradeoffs. In addition, Latta et al. (2012) compared salt tolerance between *Daphnia* populations derived from habitats with fluctuating salt levels (generalists), and populations experiencing low salt concentrations (specialists). They found that the specialists experienced lower survivorship in response to increasing NaCl, suggesting that the generalists possessed more genes with phenotypic plasticity which helped them adapt to the increased osmotic stress.

These studies leave certain questions about adaptive salinity tolerance in *Daphnia* unanswered. For example, how quickly can *Daphnia* populations go through the microevolutionary changes necessary to increase their survival in high salinity habitats? The objective of this study was to determine whether exposure to low NaCl concentrations over several generations would lead to an increased salinity tolerance in *Daphnia magna* in a laboratory setting. We used NaCl based on the results of a previous study by Ghazy et al. (2009) which reported that *Daphnia magna* was more sensitive to NaCl solutions than to synthetic seawater. We hypothesized that a *Daphnia magna* population that had been previously-exposed to low levels of NaCl for just 4 weeks would have significantly higher salinity tolerance than an unexposed population.

Method

Experimental animal maintenance

An initial population of 60 *Daphnia magna* was purchased from Carolina Biological Supply Company®. All *Daphnia* cultures were kept in 1.8-liter fish bowls containing commercially-available, pure spring water (Kroger®). Cultures were maintained on a 14:10 light:dark cycle, with overhead lights being kept on for 14 hours and off for 10 hours per day to simulate natural lighting conditions. Cultures were fed daily with 2 mL of *Selenastrum* suspension and 3 drops of yeast suspension. Based on rearing information provided by the Pacific Northwest National Laboratory (2010), an ambient air temperature range of 18-22°C was maintained throughout the study. Once weekly, half of the water in each culture was removed and replaced with fresh water. *Daphnia* were carefully transferred to a new fish bowl using a turkey baster before water changes were conducted.

Initial estimation of the NaCl LC_{50} in *Daphnia magna*

We first determined the NaCl LC_{50}, the concentration that is expected to kill 50% of a population of organisms, for *Daphnia magna*. *Daphnia* neonates (juveniles) were exposed to media with one of six concentrations of NaCl: 0.00 ppt, 2.30 ppt, 2.40 ppt, 2.50 ppt, 2.60 ppt, and 2.70 ppt. For each concentration, ten plastic Dixie® cups were filled with 100 ml of each treatment's respective solution. One healthy neonate was placed in each cup without food for 48 hours. At that time, each neonate was nudged with a pipet to determine if it was still living. The NaCl LC_{50} of 2.30 ppt determined by this test was used to identify an appropriate sub-lethal dose for the long-term exposure study and to test for changes in NaCl tolerance at the conclusion of the study.

Testing for increased tolerance to NaCl following long-term exposure

To determine whether *Daphnia magna* could develop an increased tolerance to NaCl, we established two cultures: an experimental culture with 1.00 ppt NaCl (a sub-lethal dose based on the results of the LC_{50} test) and a control culture without added NaCl. Each culture had an initial population of 60 individuals. Cultures were maintained for 4 weeks using the rearing methods described above. In the experimental culture, NaCl concentrations were increased by 0.2 ppt weekly, and reached 1.6 ppt NaCl in the final week. After 4 weeks, 40 *Daphnia magna* neonates were removed from both the control and experimental cultures and individually placed into a plastic cup with 100 ml of 2.30 ppt NaCl solution. Every 8 hours for 48 hours, the number of individuals surviving was determined for both groups by nudging each individual with a pipet.

Results

We used a log-rank test to compare survival between the control and experimental groups. This involved comparing the number of neonate deaths in each 8-hour interval over the 48-hour test period. The null hypothesis predicted no difference between groups in the probability of death in each 8-hour period (see Bland and Altman, 2004, for a description of the test). Chi-square goodness of fit tests were then used to determine if the number of *Daphnia* surviving after 48 hours in each group differed significantly from the number predicted by the LC_{50} test. The null hypothesis predicted that 20 of the 40 neonates in each treatment would die. We used 0.05 as the level of significance in all tests. If our P-value is greater than or equal to 0.05, then we would fail to reject the null hypothesis. If the p-value was less than 0.05, then we would reject the null hypothesis and conclude that survival significantly differed between the two groups based on NaCl pretreatment and salinity tolerance adaptation.

The 48-hour NaCl LC_{50} for *Daphnia magna* neonates was estimated to be 2.30 ppt based on an initial exposure to concentrations ranging from 0.00 to 2.70 ppt NaCl (Figure 1). During the 4-week exposure period, we observed that some sexual reproduction occurred in the salt-exposed population, as evidenced by the dark ephippia eggs present in the brood chambers of some females. Following 4 weeks of exposure to increasing, but sub-lethal concentrations of NaCl, neonates in the experimental group had a significantly lower death rate across 48 hours of exposure to 2.30 ppt NaCl, compared to those removed from the control group (LR = 7.5, df = 1, $p < 0.05$; Figure 2). The number of neonates taken from the control group that survived to the end of the 48-hour exposure to NaCl at the LC_{50} concentration did not differ significantly from 50% (χ^2 = 1.6, df = 1, $p > 0.05$). However, the number from the experimental group that survived to the end of the 48-hour test was significantly higher than 50% (χ^2 = 6.4, df = 1, $p < 0.05$).

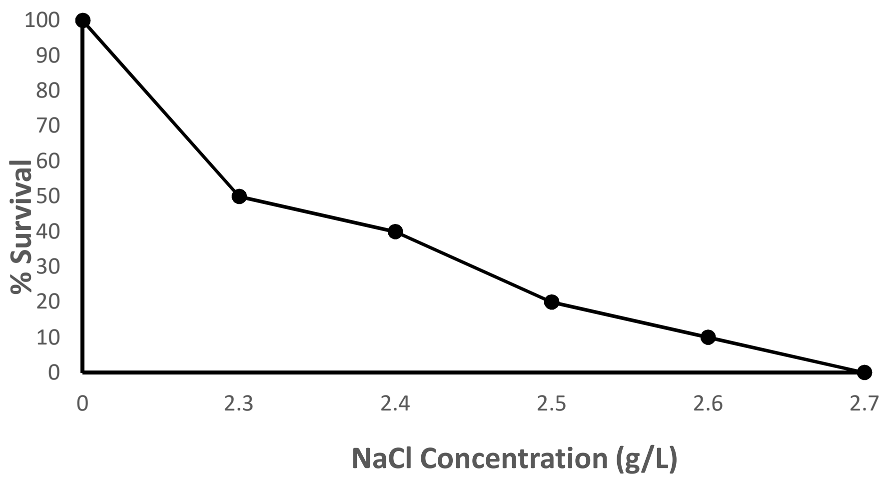

Figure 1. Percent survival of *Daphnia magna* neonates for all NaCl treatment groups. After 48 hours of exposure, 2.30 ppt NaCl was determined as the most accurate estimate of the LC_{50}.

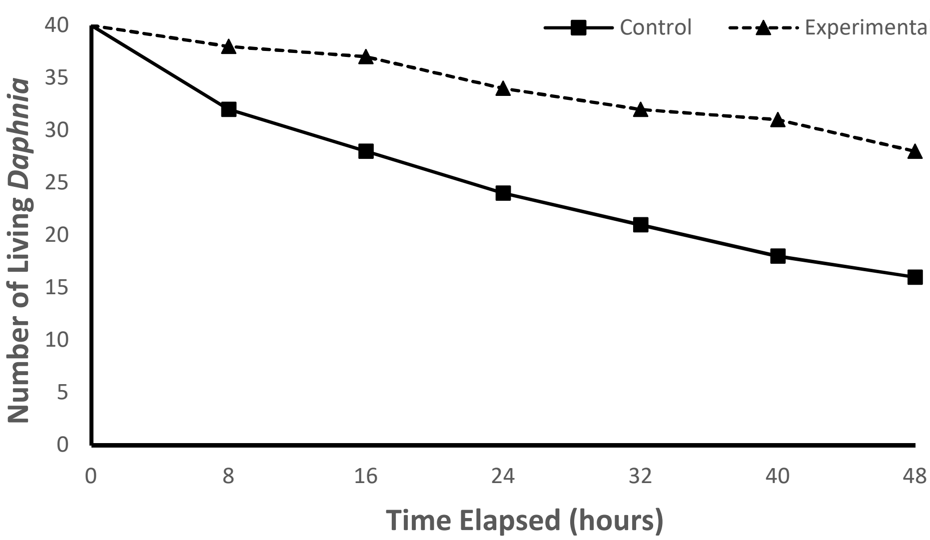

Figure 2. Survival of *Daphnia magna* neonates from the experimental and control groups exposed to a 2.30 ppt NaCl solution over 48 hours. Neonates in the experimental group had been exposed to increasing levels of NaCl over 4 weeks prior to the test, whereas those in the control group were exposed to a 0.00 ppt NaCl solution. There was a significant difference in survival between groups (LR = 7.5 df = 1, $P < 0.05$) across this time. At 48 hours, survival in the control group was not significantly different from the value expected (20 neonates, or 50% of the 40 initial neonates) by the LC_{50} (χ^2 = 1.6, df = 1, $p > 0.05$), but survival in the experimental group was significantly higher than the expected value (χ^2 = 6.4, df = 1, $p < 0.05$).

Discussion

Daphnia magna neonates removed from a population exposed to sub-lethal levels of NaCl for 4 weeks demonstrated significantly greater salinity tolerance than neonates from an unexposed population, supporting our original hypothesis. We concluded that *Daphnia magna* can evolve an increased tolerance to rising salinity levels over several generations. There are at least two possible explanations for the different levels of salt tolerance: (1) the experimental population developed greater tolerance through natural selection, or (2) individuals in the experimental population had physiologically acclimatized to salt stress before being placed in the LC_{50} concentration. Individuals in the final generation of the experimental group may have exhibited greater salinity tolerance simply because they had been previously-exposed to saline waters, unlike control individuals. These two explanations are not mutually exclusive.

Although *Daphnia magna* primarily reproduce asexually, there may have been enough genetic variation amongst the initial 60 individuals for natural selection to proceed. In addition, the dark ephippia eggs observed in the treatment group indicate that sexual reproduction was occurring, which would provide more genotypic variation for natural selection to work with. As previously-noted, Pennak (1989) described how *Daphnia* are known to reproduce sexually in response to adverse environmental conditions to promote genetic variation in the population. In this study, the high salinity conditions in the experimental group may have induced sexual reproduction. In any case, the NaCl stressor likely caused individuals lacking adaptive genotypes to drop out and individuals with adaptive genotypes to be selected for, shifting allelic frequencies in the population, resulting in phenotypes that could better tolerate the salt stress. Thus, even if asexual reproduction dominated in the population after these microevolutionary changes occurred, these genetic clones of their parent would still express the adaptive alleles for genes involved in tolerating high salinities.

Several previous studies have suggested that *Daphnia* populations can adapt to environmental stressors in similar ways. For example, Gustafsson and Hansson (2004) found that *Daphnia magna* were able to develop an increased tolerance to cyanotoxins over several generations in a 4-week laboratory exposure to increasing concentrations, similar to the one described here. Likewise, Weider and Hebert (1987) found that *Daphnia pulex* clones (defined as a "multi-locus genotype") found predominately in high salinity ponds near Hudson Bay experienced higher survival than clones derived from low salinity ponds when exposed to high salt concentrations in a laboratory setting. *Daphnia* have also demonstrated a capacity for acclimatization, where individual organisms gradually undergo physiological adjustments to adapt to environmental changes. Yampolsky et al. (2014) found that *Daphnia magna* from cultures that had been previously acclimatized to 28°C water were able to stay active significantly longer in 37°C water than individuals from cultures that were kept in 20°C conditions. A similar study by Zeis et al. (2004) investigated how providing time for thermal acclimatization affected the optimum temperature range for swimming in *Daphnia magna*. They found that increasing acclimatization temperature by 10°C (up to 30°C) resulted in a subsequent 10°C increase in the maximum thermal tolerance of tested *Daphnia*. Findings such as these suggest that adaptive tolerance in *Daphnia* may be more rooted in their phenotypic plasticity than in genetic changes. However, more research need to be conducted to determine if *Daphnia* salinity tolerance is increased via acclimatization as well.

Salinity tolerance in *Daphnia* is crucial due to the clear need for zooplankton to adapt to increasing salt concentrations, a result of the application of road salts and increased saltwater intrusion. For example, Corsi et al. (2010) found that 38% of water samples from a creek in Milwaukee killed all tested *Ceriodaphnia dubia* due to excessive NaCl concentrations. The authors attributed these high NaCl concentrations to urban runoff of road salt from the surrounding area. Brown and Yan (2015) also found that elevated chloride levels from urban runoff significantly reduced neonate production in *Daphnia* from temperate lakes in Canada. However, the effect of elevated chloride levels on *Daphnia* mortality was contingent upon food availability. Regarding saltwater intrusion, Gonçalves et al. (2007) found that *Daphnia magna* from freshwater habitats along coasts with higher salinities were more tolerant of high NaCl concentrations than *Daphnia longispina*, a species found in more inland freshwater systems with lower salinities. The authors suggested that the biodiversity and trophic structure of coastal freshwater ecosystems will likely experience significant shifts in response to saltwater intrusion into these habitats.

Future studies should involve a further investigation of the development of salt tolerance in *Daphnia* using clonal populations. This would involve taking a single, asexually-reproducing female and developing a colony made solely of her offspring, making it possible to assume that all members of the population had the same genetic makeup, barring mutations. A future study could involve developing several clonal populations and acclimatizing half of each to higher salt concentrations in a single generation. Every population could then be exposed to the NaCl LC_{50}. If the acclimatized populations had greater survival than control populations, then increased salinity tolerance could in part be explained by a process of acclimatization rather than natural selection alone. Another avenue for future work could be an exploration of the extent to which salt stress induces sexual reproduction in *Daphnia,* as we observed anecdotally in this study. Finally, future studies could involve varying study periods. By extending or reducing the exposure period, it may be possible to determine how rapidly *Daphnia* populations might adapt to salt stress by natural selection.

References

Antal, L., Halasi-Kovacs, B., & Nagy, S. A. (2013). Changes in fish assemblage in the Hungarian section of River Szamos/Somes after a massive cyanide and heavy metal pollution. *North-Western Journal of Zoology*, *9*, 131-138.

Bailey, S. A., Duggan, I. C., van Overdijk, C. D., Johengen, T. H., Reid, D. F., & MacIsaac, H. J. (2004). Salinity tolerance of diapausing eggs of freshwater zooplankton. *Freshwater Biology*, *49*(3), 286-295.

Bland, J. M., & Altman, D. G. (2004). The logrank test. *Bmj*, *328*(7447), 1073.

Brown, A. H., & Yan, N. D. (2015). Food quantity affects the sensitivity of *Daphnia* to road salt. *Environmental Science & Technology*, *49*(7), 4673-4680.

Coldsnow, K. D., Mattes, B. M., Hintz, W. D., & Relyea, R. A. (2016). Rapid evolution of tolerance to road salt in zooplankton. *Environmental Pollution*, *269*, 1-7.

Corsi, S. R., Graczyk, D. J., Geis, S. W., Booth, N. L., & Richards, K. D. (2010). A fresh look at road salt: aquatic toxicity and water-quality impacts on local, regional, and national scales. *Environmental Science & Technology*, *44*(19), 7376-7382.

Cottingham, K. L., & Schindler, D. E. (2000). Effects of grazer community structure on phytoplankton response to nutrient pulses. *Ecology*, *81*(1), 183-200.

DeMott, W. R. (1982). Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina*. *Limnology and Oceanography*, *27*(3), 518–527.

Dodson, S. I. (1972). Mortality in a population of *Daphnia rosea*. *Ecology*, *53*(6), 1011-1023.

Ebert, D. (2005). *Ecology, epidemiology, and evolution of parasitism in Daphnia* (1st ed.). National Center for Biotechnology Information, Bethesda, Maryland.

Farombi, E., Adelowo, O., & Ajimoko, Y. (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. *International Journal of Environmental Research and Public Health*, *4*(2), 158–165.

Forró, L., Korovchinsky, N. M., Kotov, A. A., & Petrusek, A. (2007). Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. *Hydrobiologia, 595*, 177-184.

Ghazy, M. M. E. D., Habashy, M. M., Kossa, F. I., & Mohammady, E. Y. (2009). Effects of salinity on survival, growth and reproduction of the water flea, *Daphnia magna*. *Nature and Science*, *7*(11), 28-42.

Gonçalves, A. M. M., Castro, B. B., Pardal, M. A., & Gonçalves, F. (2007). Salinity effects on survival and life history of two freshwater cladocerans (*Daphnia magna* and *Daphnia longispina*). *International Journal of Limnology*, *43*, 13-20.

Guilhermino, L., Diamantino, T., Silva, M. C., & Soares, A. (2000). Acute Toxicity Test with *Daphnia magna*: an alternative to mammals in the prescreening of chemical toxicity? *Ecotoxicology and Environmental Safety*, *46*(3), 357–362.

Gustafsson S., & L. Hansson (2004). Development of tolerance against toxic cyanobacteria in *Daphnia*. *Aquatic Ecology*, *38*, 37-44.

Latta, L. C., Weider, L. J., Colbourne J. K., & Pfrender, M. E. (2012). The evolution of salinity tolerance in *Daphnia*: a functional genomics approach. *Ecology Letters*, *15*, 794-802.

Liu, X., & Steiner, C. F. (2017). Ecotoxicology of salinity tolerance in *Daphnia pulex*: interactive effects of clonal variation, salinity stress, and predation. *Journal of Plankton Research*, *39*, 687-697.

Ortells, R., Reusch, T. B., & Lampert, W. (2005). Salinity tolerance in *Daphnia magna*: characteristics of genotypes hatching from mixed sediments. *Oecologia*, *143*(4), 509-516.

Pacific Northwest National Laboratory. (2010). *Raising Daphnia, the Water Flea*. U.S. Department of Energy. https://ecology.pnnl.gov/Rivers/Aquarium/daphnia.asp

Pennak, R. W. (1989). *Fresh-water invertebrates of the United States: Protozoa to Mollusca*. Wiley, Hoboken, New Jersey.

Persson, J., Brett, M. T., Vrede, T., & Ravet, J. L. (2007). Food quantity and quality regulation of trophic transfer between primary producers and a keystone grazer (*Daphnia*) in pelagic freshwater food webs. *Oikos*, *116*(7), 1152-1163.

Steiner, C. F. (2004). *Daphnia* dominance and zooplankton community structure in fishless ponds. *Journal of Plankton Research*, *26*(7), 799-810.

Tonkopii, V., & Iofina, I. (2007). The usage of *Daphnia magna* as an alternative bioobject in ecotoxicology. *Alternatives to Animal Testing and Experimentation*, *14*, 565-567.

Weider, L. J., & Hebert, P. D. N. (1987). Ecological and physiological differentiation among low-arctic clones of *Daphnia pulex*. *Ecology*, *68*, 188-198.

Yampolsky, L. Y., Schaer, T. M., & Ebert, D. (2014). Adaptive phenotypic plasticity and local adaptation for temperature tolerance in freshwater zooplankton. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1776), 1-9.

Zeis, B., Maurer, J., Pinkhaus, O., Bongartz, E., & Paul, R. J. (2004). A swimming activity assay shows that the thermal tolerance of *Daphnia magna* is influenced by temperature acclimation. *Canadian Journal of Zoology*, *82*(10), 1605-161.