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Article

Effects of photoperiod alterations on stress response in *Daphnia magna*

Johnny Kelly[1], **Terese Grateful**[1]
[1] Council Rock High School North, Newtown, Pennsylvania

SUMMARY

In the modern world people are often impacted by factors that control when they can sleep and for how long, which results in an altered photoperiod. Alterations in photoperiod can occur from a variety of factors, ranging from working night shifts to travelling and changing time zones, and therefore influence the daily lives of many people. The purpose of these experiments was to determine if alterations in photoperiod affect the stress response in *Daphnia magna*. We hypothesized that if *Daphnia magna* are exposed to alterations in photoperiod, then there will be an increased stress response. We kept two distinct *Daphnia* populations and exposed the experimental group to a shorter photoperiod of 12 hours instead of the traditional 24-hour photoperiod. During the testing period, we tracked possible stress responses, including mean heart rate, brood size and male-to-female ratio. There were statistically significant differences between the control and experimental groups for both heart rate and brood size. Specifically, the experimental group exhibited an increased heart rate and brood size relative to the control group. Within the experimental group, there was statistically significant variation in the brood size over time, with the peak brood size occurring on day three or four in the seven-day trial. Male-to-female ratio did not have a statistically significant response to the altered photoperiod. The results found support the hypothesis of increased stress when photoperiod is altered, which could also indicate that altered photoperiod may have an impact on people that constantly experience changes in their photoperiod.

INTRODUCTION

Daphnia magna are a type of freshwater organism in the family *Daphniidae* and the order Cladocera, with the common name of water fleas. They are filter feeders that have a high production rate of eggs (1). Each female releases a new brood of eggs when it molts, which occurs every 3 to 4 days. *Daphnia* are a good model organism in being able to track stress response as they require little maintenance, have a high rate of reproduction and have a clear exoskeleton, which allows for the external visualization of organs (2). As *Daphnia* are a good model organism for tracking stress levels, they can be used in tracking alterations in photoperiod.

Photoperiod is the length of a day for an organism. Many organisms use their circadian rhythm, or their natural body clock, to link to the 24-hour photoperiod of the Earth, which has approximately 12 hours of light and 12 hours of dark at any one location, although this varies throughout the year. Humans experience photoperiod shifts throughout their everyday lives, whether it be due to working night shifts or traveling through different time zones, and the impact of these photoperiod alterations on stress levels in organisms is unclear. Therefore, by studying photoperiod alterations in model organisms, we can infer whether changes in photoperiod may have adverse impacts on the stress levels of humans.

In *Daphnia*, brood size (3), male-to-female ratio (4) and heart rate (5) can all be used as indicators of the stress response. Following a stress event, heart rate, brood size, and male-to-female ratio are all expected to increase due to a fight or flight response, a greater production of eggs in order to increase the chance of survival for the population, and the cyclical parthenogenesis of *Daphnia magna* as a pathway of increased reproduction (6), respectively. Cyclical parthenogenesis is a reproductive life cycle *Daphnia* use that allows for the switching between sexual and asexual reproduction between generations under high and low stress (7). Under low stress, females reproduce asexually, and only females are produced. However, when high stress is introduced to the population, the epigenetic landscape of *Daphnia*, or heritable phenotype changes that do not alter DNA, shifts genetically female *Daphnia* into males to allow for sexual reproduction to occur (8). Sex determination occurs after the egg is produced and before development begins, allowing for the *Daphnia* to express its phenotype in response to environmental stress. The increase in males and resulting sexual reproduction allows for greater genetic variation than asexual reproduction, which is favorable in high stress environments. Since sexual reproduction is promoted when the *Daphnia* are exposed to stress, the male-to-female ratio can be used to measure stress, similarly to heart rate and brood size (9). *Daphnia* males can be identified by their smaller size, larger antennules and modified legs. If alterations in photoperiod were a stressor for *Daphnia*, this would likely result in an increase in the number of males in the population over time.

In this study, we investigated the relationship between photoperiod and stress response in *Daphnia magna*. If there is an alteration in photoperiod for a *Daphnia* population, we hypothesized that there would be an increase in the stress response, which would be reflected in an increase in heart rate, brood size and male-to-female ratio. We first determined if there was a stress response seen due to a change in the photoperiod. If there was a stress response, we studied whether said stress response varied over the time that the stressor was applied. Variation over time would suggest that adaptation and acclimation to the stress was occurring. After experiments were conducted, we found an increase with heart rate and brood size due to photoperiod alterations in the *Daphnia* populations. Additionally, brood size varied over time during the stress response, with the largest brood size in the middle of the 7-day trials. Male-to-female ratio did not significantly change in response to an altered photoperiod. Changes were seen in brood size and heart rate in response to the change in photoperiod, but changes in male-to-female ratio were not significant. These results suggest that organisms demonstrate stress responses to shifts in photoperiod, with an initial reaction to the stressor and then acclimation to the stressor over time.

RESULTS

In order to determine if there was a stress response in *Daphnia magna* as a result of photoperiod alterations, we tracked three stress responses following a photoperiod alteration, heart rate, brood size and male-to-female ratio in the population and conducted three experimental trials in total. The *Daphnia magna* started in a stock population that was conditioned to a 24-hour photoperiod, with 12 hours of light and 12 hours of dark. In order to track the three stress responses, two tanks were set up alongside the stock population to hold our control and experimental populations. At the beginning of testing, the experimental groups were switched to a 12-hour photoperiod, with 6 hours of light and 6 hours of dark, while the control group continued with a 24-hour photoperiod.

To test the heart rate, four *Daphnia* were transferred to well plates for both the control and experimental groups, and their heart rate was measured daily for three days. The experimental group had the higher heart rate each day, which was greatest initially after *Daphnia* were exposed to the stressor and decreased slightly by the end of the trial **(Figure 1)**. The control group did not have any noticeable changes over time **(Figure 1)**.

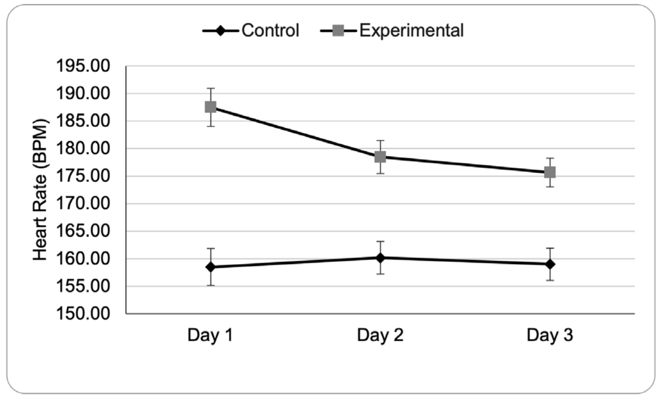

Figure 1: Mean heart rate. The average heart rate of eight *Daphnia* was determined over three trials, with four *Daphnia* in both the control (dark gray) and experimental groups (light gray). Error bars show the standard deviation for the heart rate.

In order to determine the male-to-female ratio, a sample of five *Daphnia* was taken from both populations. From these two samples, we determined the number of females. The experimental group generally had a lower average percent of females relative to the control group, with its minimum around day 4, but by the end of the trial it returned to a male-to-female ratio similar to that seen at the start of the trial **(Figure 2)**. The control group stayed constant throughout the trial **(Figure 2)**.

To find mean brood size, eggs from each female were counted. As the male-to-female ratio varied each day, there was an average of 3 to 5 females in the sample of 5 *Daphnia* to find mean brood size. The experimental group had a larger average brood size throughout the trial than the control group, and its brood size peaked around day four until returning to its original value by the end of the trial **(Figure 3)**. The control group had consistent brood sizes throughout the experiment **(Figure 3)**.

A two-sample *t*-test with a significance level of 0.05 was used to evaluate the differences between the control and experimental groups for all three stress responses. The measured heart rate had a *t*-statistic of 5.34 exceeded the critical value of 2.074. Brood size and male-to-female ratio both had a critical value of 2.064, but the brood size had a *t*-statistic of 3.14, which exceeded the critical value, while male-to-female ratio had a *t*-statistic of 1.58, which did not exceed the critical value. These results suggest that alterations in photoperiod caused statistically significant changes in the heart rate and brood size of *Daphnia*, but not in male-to-female ratio.

A repeated measures ANOVA test was conducted with a confidence level of 0.05 to determine if there was variation over time for the three stress responses. We found that the

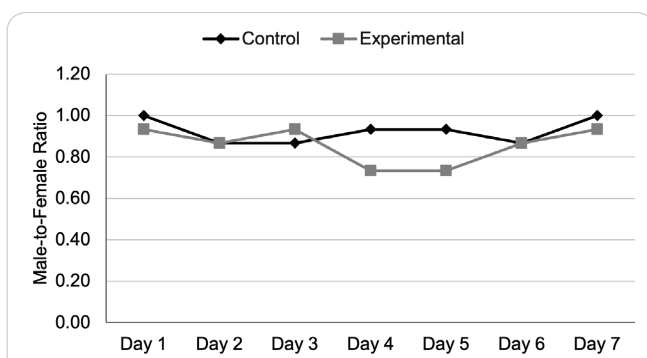

Figure 2: Male-to-female ratio. The average male-to-female ratio for three seven-day trials was taken, with the average male-to-female ratio being determined by taking a random sample of 5 *Daphnia* from the population. The error bars represent the standard deviation.

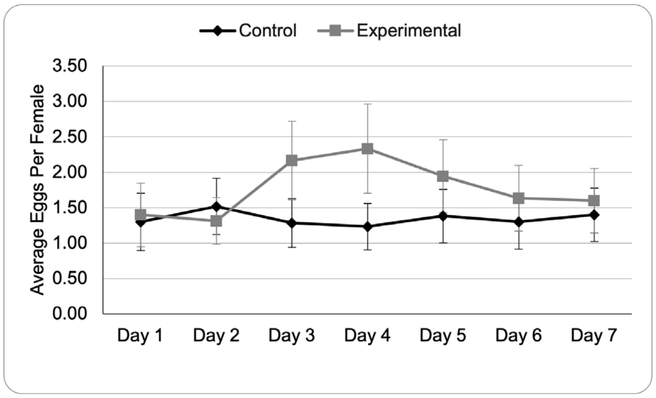

Figure 3: Mean brood size. The average number of eggs per female *Daphnia* in a random sample of three to five females each day in three trials over seven-day periods were counted, and the error bars represent the standard deviation.

heart rate did not have significant changes in either the control or experimental, with the control group having F(2,4)=0.05735 and the experimental group having F(2,4)=0.21204. Neither of these F statistics exceeded the critical value of 6.94. For the control group of brood size, the repeated measures ANOVA presented with F(5,10)=0.46773, which did not exceed the critical value of 3.33 at a significance level of 0.05. However, the experimental group did have statistically significant variation over time with F(5,10)=4.01277, which exceeded the critical value of 3.33 at a significance of 0.05. For the male-to-female ratio, the control had F(5,10)=1.34078 and the experimental had F(5,10)=2.08050, and neither of which exceeded the critical value of 3.33. These results suggest that for the control and experimental groups of the heart rate and male-to-female ratio as well as the control group of the brood size experiment, there was not statistically significant change in the levels of stress indication over time. For the experimental group of the brood size experiment, there was statistically significant change in brood size over time.

DISCUSSION

In this work, we sought to determine if exposure to altered photoperiods in *Daphnia magna* resulted in a stress response. Both the association between the altered photoperiod and stress response as well as the change in the stress response over time were tested during our experiments. Our findings provide evidence that altered photoperiods do in fact cause a stress response in *Daphnia*. Both heart rate and brood size showed increased indications of stress when exposed to an altered photoperiod. However, male-to-female ratio did not show significant differences in its response to photoperiod alterations but still showed the expected response, with the experimental group having more males in the sample. Additionally, analysis of the variation over time for brood size showed that the *Daphnia* had an initial spike following the onset of photoperiod stress but later became acclimated to their new photoperiod.

There were several possible sources of error during our studies. Firstly, error may have been generated from the use of separate tanks to maintain *Daphnia* populations. Although they were kept in similar conditions, they were kept in separate rooms in order to avoid the photoperiod of each tank affecting the other. There could have been slightly different environmental conditions that affected the stress response. For the number of females and number of eggs measurements, a small sample of *Daphnia* was taken from each population, so there might have been variation from the true population mean with the samples taken each day. Sample sizes were also variable across the different measured parameters. Testing for the male-to-female ratio resulted in only one data point each day, which was how many females were in the sample of five *Daphnia*, while heart rate and brood size had three to five data points each day. For change over time analysis of heart rate, the *Daphnia* in each well plate only survived for three days, so the data was limited to only three days. Since there was such a low number of days to investigate variation each day, either the number of *Daphnia* would need to increase or the number of days that the heart rate of the *Daphnia* was tracked would need to increase in order to increase our confidence in these findings.

In the future, the effects of photoperiod alterations on stress responses can be further evaluated through expansion into higher organisms, including humans. Experiments can be done on organisms that have greater genetic similarly to humans as well as photoperiods and circadian rhythms similar to that of humans in order to better predict outcomes in human subjects. On top of this, observational studies can be done to determine if people that have frequent shifts in their photoperiod have higher levels of stress.

The results of this study show that photoperiod alterations have an impact on the stress response in *Daphnia*, with brood size and heart rate both showing significant increased stress indication. Brood size also showed significant variation over time, as the *Daphnia* had an initial spike following the onset of stress but became acclimated to the stress by the end of the seven-day trial. The stress response seen in daphnia following a change in photoperiod lead us to anticipate a similar impact on higher order organisms, such as humans. In order for the same relationship between photoperiod alterations and stress response in humans to be established, further experiments and studies would need to be conducted relating to organisms more similar to humans or on humans themselves.

MATERIALS AND METHODS

One-gallon tanks were used and filled with a third to half a gallon of spring water. An air pump, tubing and air stone were used to aerate the water. Tubing valves were used to prevent turbulent water flow in the tanks. Water was aerated for two days prior to transferring the *Daphnia* into the tanks. *Daphnia magna* from Aqua L'amour were used and were allowed to recover from shipping trauma for at least 24 hours before they were transferred into their tank. A pipette was used to transfer

Daphnia.

The pH was regulated with API Proper pH7 buffer to achieve a pH of around 7. The pH was tested once a week. *Daphnia* were fed once every week with Carolina *Daphnia* Food. Desk lamps were set up on timer outlets in order to be able to control the photoperiod of the *Daphnia*. Both populations were initially exposed to a 24-hour photoperiod with 12 hours of light and dark. The experimental group was changed over to a 12-hour photoperiod with 6 hours of light and dark when the experiments began. *Daphnia* were placed into well plates for the heart rate measurements.

For each trial, a sample of five *Daphnia* was taken from the population of around 20 to 30 *Daphnia* in order to track the brood size and male-to-female ratio. The number of females was recorded as well as the number of eggs that each female had. After obtaining the necessary data, the *Daphnia* were then returned to the tanks. Because the exoskeleton of *Daphnia* is clear, the eggs were visible through the skin as dark spheres. Testing was repeated once a day for 7 days.

Using a microscope, the heart rate of the *Daphnia* in the well plates was determined by counting three consecutive 10-second intervals for four *Daphnia* each day The heart rate was tracked for 3 days, as this was the average amount of time the *Daphnia* survived in the individual well plates.

After collecting data, two statistical tests were conducted manually in excel. A two-sample *t*-test with a significance level of 0.05 was conducted to determine if there were differences in the stress response of the *Daphnia* between the control and experimental groups. A repeated measures ANOVA test was conducted with a significance level of 0.05 to find if there was a difference between the stress response of the *Daphnia* over time by comparing the dependent variable each day. Both statistical tests were conducted for heart rate, male-to-female ratio, and number of eggs.

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