International Journal of Applied Environmental Sciences
ISSN 0973-6077 Volume 11, Number 4 (2016), pp. 1037-1048

http://www.ripublication.com

Evaluation of Chemotaxis in *Daphnia magna* in a Multitasking Lake Model

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Abstract

Daphnia magna has been the subjects of study in many areas of the genome study to safety pharmacology. Further, the application of Daphnia model has been widely accepted to be useful in ecotoxicological disciplines. However, the study of horizontal migration was not easy to find. Also, its application might extend to the field of bioassay technology if confirmed its sensitivity of parameters to differentiate chemical compounds and concentrations. This study was carried out with an assumption of contamination in a single-source chemical-exposed lake. A lake model was designed based on the miniaturized representation of gradual diffusion of chemicals, in this case, an insecticide with an active ingredient deltamethrin, and an herbicide with an active ingredient: 2, 4-Dichlorophenoxyacetic acid. The standard and chemical-affected heart rates of the *D. magna* were determined after 30-minute incubation periods. Using these concentrations determined by the preliminary study, the *D. magna* were placed in the lake model at a releasing point and subsequently diffused with these chemicals from a source point. The positional values of *D. magna* were recorded at every 20 minutes with a numbering coordinate system consisting of evenly divided grids in the lake model for 140 minutes. The parameter sum distance was calculated with the sum of each point values located at each sampling time point. The result of the study with and without the insecticide and herbicide showed correlations between the chemotactic behavior of *D. magna* and the chemicals. The results indicated a negative chemotactic response to the insecticide and positive chemotactic behavior to the herbicide.

Keywords: *Daphnia magna*, chemotaxis, aquatic toxicity, bioassay, migration, sum distance

INTRODUCTION

Many bioassay techniques have been established in the last couple of decades for relieving a growing concern that the levels of chemicals and wastewaters should be controlled before they are released into environmental ecosystems. (Akcil & Koldas, 2006; Le et al, 2010) The task of assessing the effects of toxic substances involves the monitoring of complex biological systems, at both the individual and community level. (Van der Oost, Beyer & Vermeulen, 2003) *D. magna* are commonly used in eco-toxicological and pharmaceutical studies. (Fent, Weston & Caminada, 2006) There are existing studies that observe the effects of chemicals like insecticides and pesticides on *D. magna* for diverse invertebrate models. (Holmstrup et al, 2010) Some have studied the effects of chemicals on the heart rates of *D. magna*, while others have studied the vertical migration in response to the presence of light and predatory smells. Vijverberg, Kalf & Boersma, 1996; Boyd, Brewer & Williams, 2002) For example, one study states that the presence of fish smell increased the fraction of the *D. magna* population exhibiting fast swimming after a change in light. (Dodson, Tollrian & Lampert, 1997) Yet, no published articles have been found for quantitative studies, and no study has scrutinized the horizontal chemotactic properties of *D. magna* at locations where the contaminated chemicals exist, such as lakes and ponds. This experiment attempts to investigate the existence of the horizontal chemotactic ability of *D. magna* through the creation of a Lake Model – one in which the chemicals diffuse from a single source, as they may in a real lake. A lake model consisting of six lanes was created and assembled using acrylic boards to demonstrate the slow diffusion of a solute in water over time. This concept deemed important because of the phenomenon called run-off. When soil is infiltrated to its full capacity, excess water flows over the land into bodies of water and along the way, and the water may pick up contaminants like the insecticides and pesticides used in this study. When the water is deposited in a body of water, the contaminants present from the land is also stored in the water. These processes that occur in bodies of water may have great impacts on the aquatic organisms living in the water like *D. magna*. (Fatta-Kassinos, Vasquez & Kümmerer, 2011). This study also introduces another phenomenon called bio magnifications. In biomagnifications, the concentration of a substance increases as it travels through the food chain and this affects food chains in all ecosystems. (Jardine, Kidd & O’Driscoll, 2013) Although the levels of the chemicals used in the study are believed to be significantly higher than those found in real lakes, the results of this study may contribute to the understanding of the behavioral relationship between chemical contamination and organisms in a food chain, like *D. magna*. The objectives of the study were to examine the existence of chemotactic property expressing by horizontal migration, and further, if existed, to evaluate its relation with different concentrations.

MATERIALS AND METHODS

A. Manufacturing Multitasking Lake

The multitasking lake was assembled based on preliminary data on the diffusion rates of chemicals and previously published archives of real eco-stable lakes. The dimensions were determined with the consideration of the following three factors involved, i.e., the estimated swimming pattern, the chemical's diffusion rate and study duration. Multiple 3-inch wide strips of 3 mm-thick acrylic boards (Acropan, NY) were prepared with an acrylic knife and fixed in place with High Performance General Purpose Clear Silicone Adhesive Sealant (All-Spec, NC) to create a rectangular water tank of 18 inches wide and 24 inches long as in Fig. 1.

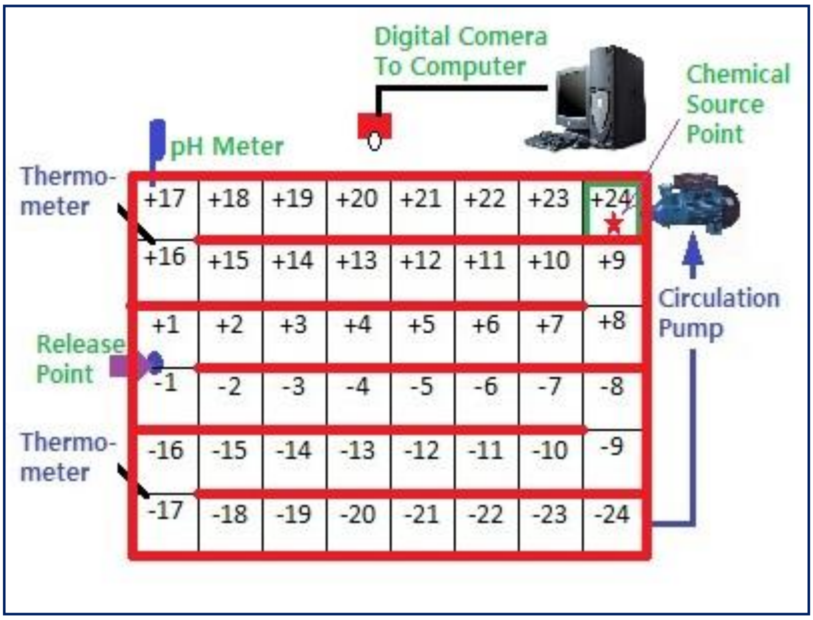

Figure 1: A Diagrammatic Illustration of the Multitasking Lake Model

The six inside lanes were designed to be 3 inches wide, and each 3 inches by 3 inches' square grids was drawn at the back of the transparent acrylic board, and serial numbers were written for a coordinate of the numbering system. A center point called the *D. magna* Release Point marked, and each square was numbered with a positively ascending orientation toward the chemical source, while negatively descending toward the opposite end. This numbering system introduced a quantitative measurement to assess the chemotactic properties to each group of *D. magna.* An infusion mini-DC water pump (Lightobject, CA) was installed on the side of the lake model, with which the water current in more natural lakes could be simulated by inducing the circulation of water from the source to the end point of the lake with a DC voltage controller with Tekpower DC Variable Power Supply (Kaito Electronics, Inc, CAY). A digital webcam microscope (DinoCapture, CA) was installed to visualize and record the locations and swimming patterns of *D. magna* if needed. The water temperature was monitored with thermometers, while a pH Hanna Instruments HI 98107 Tester (Small Parts, WA) was used to confirm the movement of chemicals in the lake model.

B. Materials and Reagents

D.magna were purchased from Blue Spruce Scientific Supply (Boulder, CO). The organisms were used at least three days after being acclimated to a tank filled with filtered water. The *D. magna* were fed with active yeast and algae as needed. The variations in size of the *D. magna* were carefully observed, and mostly medium-sized *D. magna* were chosen as the optimal population for the study.

The insecticide Bayer Advanced Carpenter Ant & Termite Killer Plus was purchased from the Vandervilt (New City, NY), had an active ingredient of deltamethrin comprising of 0.02% of the insecticide, while all other ingredients labeled as inactive. Deltamethrin belonged to a class of chemicals called pyrethroids. It was known to have a high toxicity to fish under laboratory conditions.

The herbicide Ortho Weed B Gon we employed had an active ingredient of 2, 4-Dichlorophenoxyacetic acid (2, 4-D). 2, 4-D was a commonly used herbicide that interfered with normal plant growth processes. 2, 4-D made up 0.12% of the herbicide, while other non-active ingredients occupied the rest.

C. Heartbeat Measurements

a. Normal Heart Rate

The normal heart rate of *D. magna* was measured to verify the physiological uniformity of heart rates considered within an acceptable range of cardiac functionality. In this respect, *D. magna* was transported onto a concave microscope slide which was placed on a compound light microscope. A concave *D. magna* holder in the center of the microscope slide was created with a micro-drill, which facilitated the *D. magna* to stay in the viewing window, while preventing continuous movements and remaining in the water. The number of seconds it took to count 20 heartbeats was timed. The number of heartbeats in one minute was calculated by applying the relationship of 60 seconds and one minute. And, the heart beat rate was determined with 28 *D. magna* for statistical significance.

b. Heartbeats at Various Diluted Solutions

The optimal levels of chemical effects on *D. magna* were needed to be identified first to study the horizontal response of them toward the chemical gradients in the lake model. Serially diluted solution was prepared: Seven test tubes were obtained and labeled accordingly. Approximately two mL of the insecticide was placed in the first test tube. And, beginning from the second to the seventh test tubes, 1.8 mL of water was added to each test tube. The insecticide of.0.2 mL from the first test tube was transferred to the second test tube and shook thoroughly. And, subsequently, 0.2 mL of the mixed solution was transferred to the third test tube, and this process continued up to the seventh test tube. This procedure created the ratio of the chemical mixture to water as dilution factors from $1:10^1$ and up to $1:10^6$. A single *D. magna* was dropped into each test tube after a normal heart beat rate was measured and waited for 30 minutes. After the incubation period, the Daphnia was picked from the tube and placed onto the slide. The heartbeat of *D. magna* was counted and calculated in the same way the *Normal Heart Rate* was determined earlier. This was repeated for statistical purposes and with the herbicide as well. The goal of this measurement was to determine the optimal concentrations at which the *D. magna* did not die but were affected by the chemicals to be referenced in additional studies.

D. Evaluation of Relative Sum Distance

a. Measuring Sum Distance in Lake Model with No Chemicals

The lake model was filled with 16, 300 cm^3 of filtered water for every trial. The quantity of water was determined by marking a couple of signs at an identical height around, to which the model was filled with water to be up to 70% of its full capacity. First, ten *D. magna* were placed at the designated Release Point. Every twenty minutes after the *D. magna* were released, the located coordinate of the organisms was recorded for 140 minutes. The sum distance of the *D. magna* was calculated and recorded respectively, by taking the sum of the specific locations of the ten *D. magna* at a particular time. This measurement was repeated for the additional experiment. The purpose of this evaluation was to confirm that the *D.* magna would distribute randomly without experimental bias in the lake model.

b. Calculating Sum Distance in Lake Model with Insecticide and Herbicide

The lake model was filled with filtered water up to the signs of the water level. Ten *D. magna* were carefully released at the Release Point. Thirty minutes after the *D. magna* were placed into the model, 163 mL of the insecticide was added into the Source Point, which was located at the end of the positive coordinate as in Fig. 1. When chemical added, a teabag effect was achieved by using 250 mL polyethylene container with thirty holes of 1.0 mm diameter perforated around and by slowly adding the compound to the container. This allowed the chemical to diffuse at a controlled diffusion rate. The volume of the chemical was determined using the results from *Heartbeats at Various Diluted Solutions*, because when the insecticide was added into the Model, this created at 1:100 ratio of the chemical to the solution. The 1:100 ratio, or 10^2 dilution factor, is a concentration at which the heart rates of the *D. magna* are affected by the chemical. The locations of the *D. magna* were recorded at twenty minute intervals after the original thirty minutes for 140 total minutes. This was then repeated using 326 mL of the insecticide to create a 2:100 ratio of the chemical to the solution. This experiment was repeated for statistical purposes and with the addition of the herbicide in place of the insecticide.

RESULTS

Normal Heart Beat Rate Estimation

The heart beat rates of 28 *D. magna* were determined using the method described above. The heart beat rates ranged from 240 beats per minute to 440 beats per minute. Within this data, the average heart rates ranged from 320 beats per minute to 350 beats per minute. All of these values could be considered to fall within the normal range of heart rates of *D. magna* when compared to previously published data (Lovern, 2007). The heart beat rate assessment of experimental populations gave us the verification of the uniformity of study group employed in the study. For example, no adult daphnia with hatched eggs on its back was included in the study.

Heart Beat Evaluations at Various Diluted Solutions

As seen in both Figures 2 and 3, the *D. magna* died in the solutions with a dilution factor of $1:10^0$, or in the original chemical concentration. The heart rates at levels with dilution factors of $1:10^4$, $1:10^5$, and $1:10^6$ for both the insecticide and herbicide were not affected by the chemical, considering that at these values approaching the plateau, the heart rates were consistent and not significantly changed compared to the average numbers. At the concentrations with dilution factors of $1:10^1$ and $1:10^2$, the heart rates of the *D. magna* are being significantly affected. As mentioned previously, the purpose of this experiment was to determine the optimal concentrations of the chemicals to be used in additional studies. The optimal concentration of the chemicals defined as the levels at which the *D. magna* were affected by the chemicals, based on their heart rates, but did not necessarily die. These levels determined as those with dilution factors of $1:10^1$ and $1:10^2$.

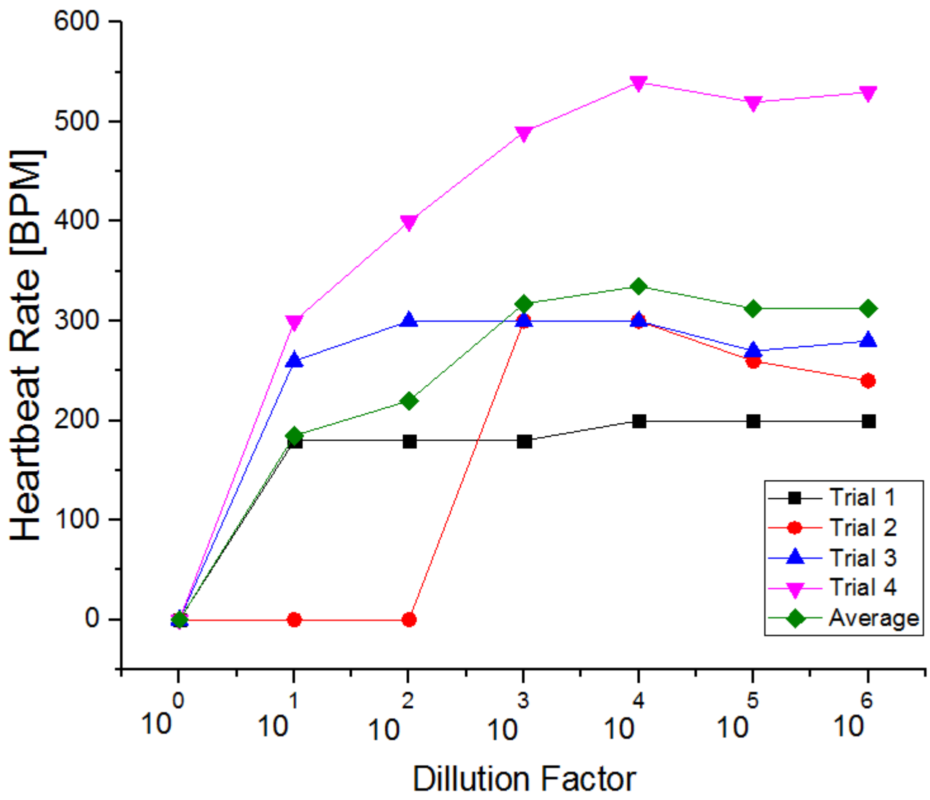

Figure 2: Heart rate of 28 *D. magna* in dilutions of factors of ten of Bayer Advanced Carpenter Ant & Termite Killer Plus ® Insecticide

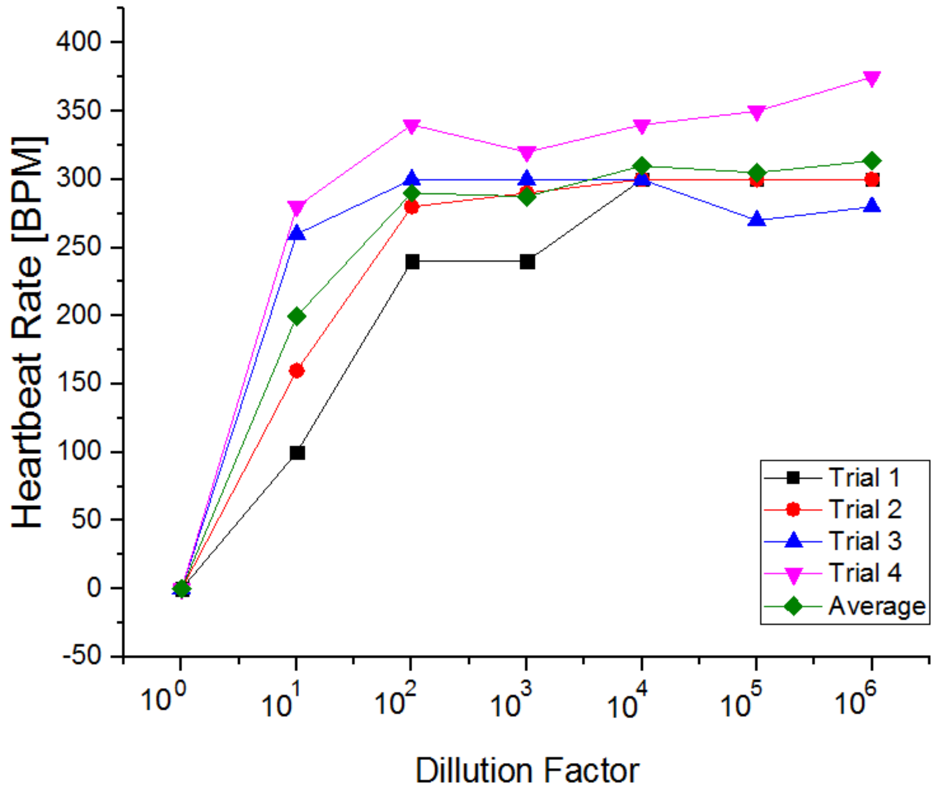

Figure 3: Heart rate of 28 *D. magna* in dilutions of factors of ten of Ortho Weed B Gon ® Herbicide

Calculating Sum Distance in Lake Model with No Chemicals

Fig. 4 shows the changing pattern of the sum distance. The four individual trials gave us the insight on how the gradual dispersion of *D.magna* with time should be summarized. Over the one hour, some experiments presented more positive sum distances, and other trials had more negative sum distances. Specifically, trial 3 displayed a larger more positive sum distance, while trial 2 displayed a more negative sum distance. However, over the course of the hour, at each of the ten-minute intervals, the average sum distance among the four separate trials was nearly close to the baseline. This meant that the *D. magna* might have traveled towards the more positive end of the lake model or the more negative end of the lake model, but because the average is very close to zero, the *D. magna* traveled without bias through the model. The data showed that any temporal variations could be eventually averaged out with the number of experimental trials increased.

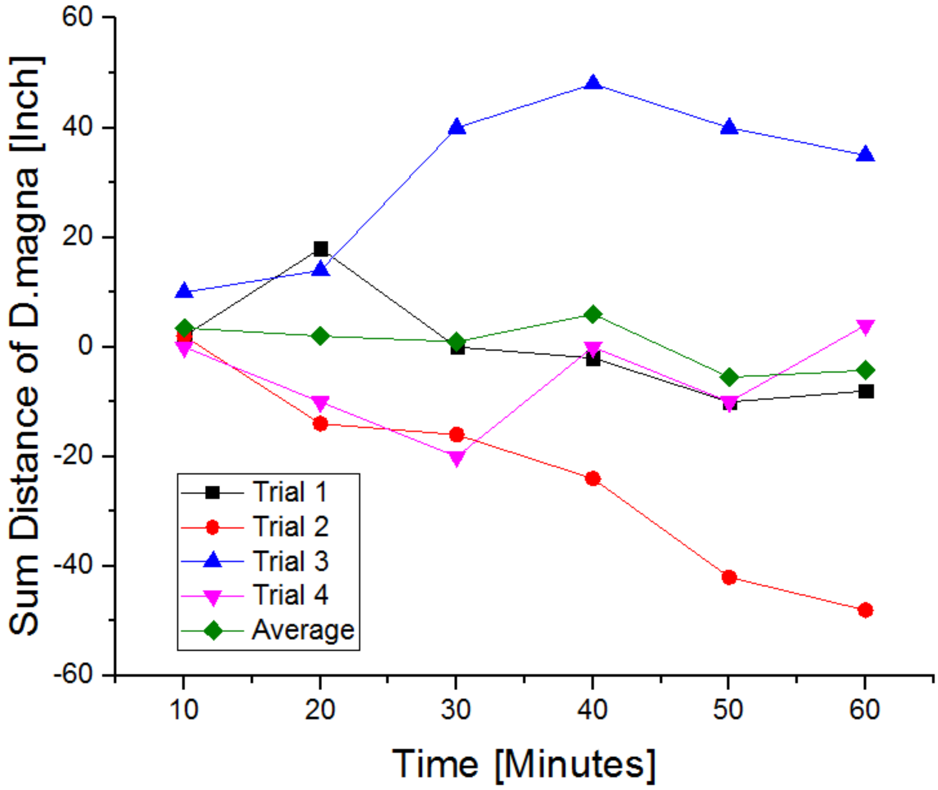

Figure 4: Sum distance of 10 *D. magna* per trial in Lake Model

Sum Distance Calculation in Lake Model with Insecticide and Herbicide

Table 1 tabulated one of our worksheet filled with equal numbers of each D. magna at scheduled sampling time. The sum distance of the *D. magna* calculated by adding all the coordinate numbers of the specific locations of the ten *D. magna* at a given moment. The sum distances of the *D. magna* at every 20 minutes over 140 minutes after the addition of either the insecticide or herbicide are shown in Figures 5 through 8. As the y-axis indicates, the adjusted sum distance is graphed. In order to best represent the change in locations of the *D. magna* in relation to time, the sum distance at each of the 20-minute intervals has been modified by the sum distance found at the time of 0. This better shows the difference of the sum distances over the course of the study. As shown in Figure 5 the trend lines have negative slopes, indicating that over the 140 minutes, the final sum distance was more negative in comparison to the sum distance at the time of 0. This means that the *D. magna* traveled toward the greater negative numbers, or away from the insecticide source. On the other hand, according to the positive slopes of the trendlines in Figure 6, during the study period, the *D. magna* traveled toward the greater positive numbers, or toward the herbicide source. There was no conclusive data acquired on the change of chemotaxis, which would be studied in the future.

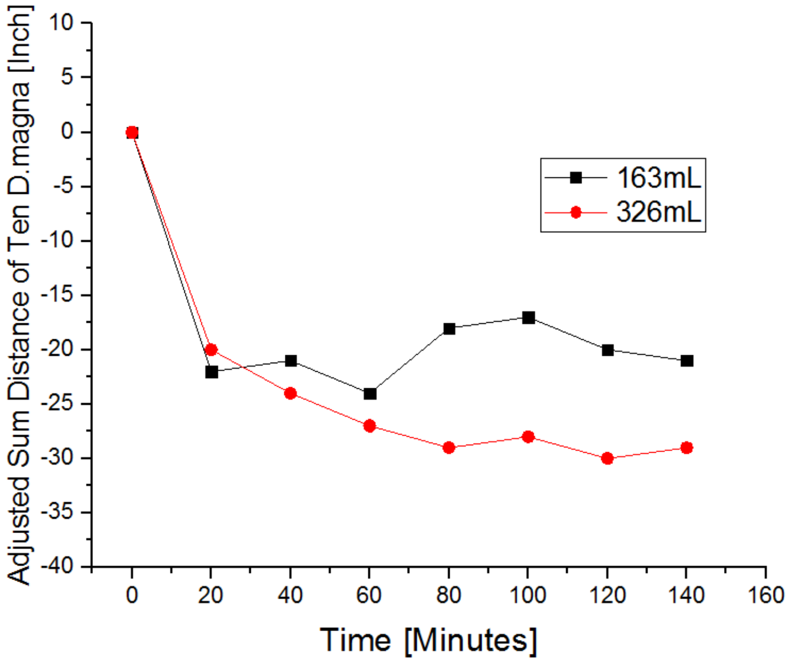

Figure 5: Adjusted sum distance of ten *D. magna* per trial with 163 mL insecticide over 140 minutes and adjusted sum distance of ten *D. magna* per trial with 326 mL insecticide over 140 minutes

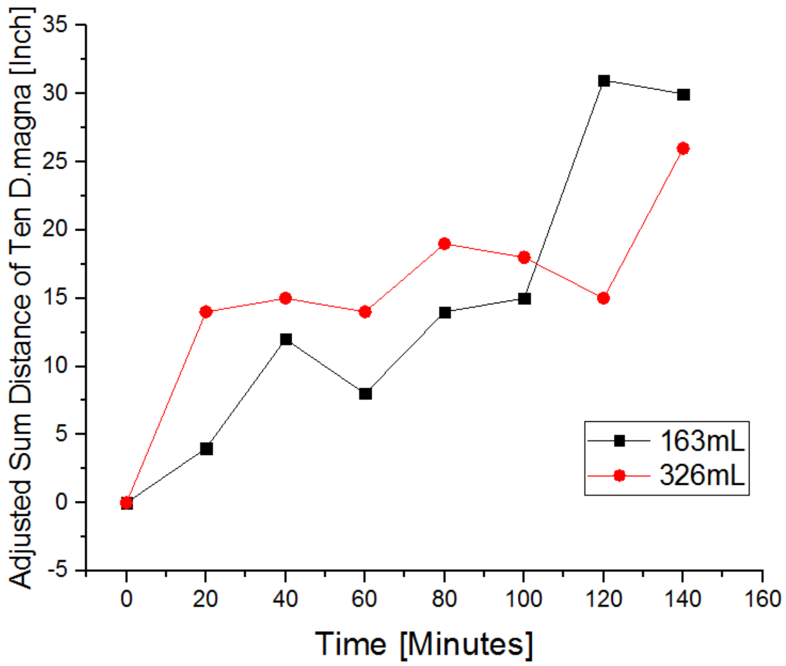

Figure 6: Adjusted sum distance of ten *D. magna* per trial with 163 mL herbicide over 140 minutes and adjusted sum distance of ten *D. magna* per trial with 326 mL herbicide over 140 minutes

Table 1 represents one example study sheet marked with coordinate numbers at which *D. magna* are located. The sum distance was calculated by adding all the numbers at any specific time. The adjusted sum distance (ASD) was obtained by adding or subtracting the Sum Distances by the sum distance at time 0. Note the number of Daphnia D#1, D#2 and up to D#10 do not necessarily represent specific *D. magna.*

Table 1: An Illustrative Worksheet from the study of chemotactic properties.

Time (min)	Numbers read from each grid coordinate at scheduled time points										**Sum Distance**	**ASD**
	D.#1	D.#2	D.#3	D.#4	D.#5	D.#6	D.#7	D.#8	D.#9	D.#10		
0	+1	+1	+1	+1	+1	+1	+1	+1	+17	-2	+**23**	**0**
20	+17	+17	+3	+1	+1	+1	+1	+1	-9	+4	+**37**	+**14**
40	+17	+17	+17	+4	+1	+1	+1	-5	-9	+11	+**55**	+**32**
60	+19	+17	+6	+4	+1	+1	+1	+1	+1	-8	+**43**	+**20**
80	+17	+17	+17	+8	+4	+1	+1	+1	-8	+3	+**61**	+**38**
100	+17	+17	+17	+7	+8	+2	+2	+1	+1	-9	+**63**	+**40**
120	+17	+17	+17	+15	+5	+1	+1	+1	+17	-7	+**84**	+**61**
140	17	17	17	17	17	1	1	1	-1	-8	**+79**	**+56**

DISCUSSION

According to the first two preliminary studies, or Figures 2 to 3, there was a general indirect relationship between the concentrations of the insecticide and herbicide and the number of heartbeats of the *D. magna*. This indicated that in higher concentrations of the specific chemicals, the number of pulses of the *D. magna was* fewer and vice versa. Using the levels at which the chemicals affected the heart rates of the organisms (the areas of the trendlines with positive slopes), the volumes of chemical to be used in the third and final studies were determined as 326 mL and 163 mL, considering the total amount of water in the lake model (16300 cm^3). The third preliminary study shown in Figure 4, showed that the overall average of the distances traveled by the *D. magna* for one hour was constant, thus allowing for the further study. The locations of *D. magna* recorded for 140 minutes, and the sum distance calculated at each of the intervals. The results for both the 326 mL insecticide and 163 mL insecticide show the negative chemotactic behavior of *D. magna* to the insecticide because of the negative-slope trendline based on the sum distance of the organisms, meaning that as the concentration of the insecticide in the water increased over time, the *D. magna* traveled away from the source. Similarly, the results for both the 326 mL herbicide and 163 mL herbicide show the positive chemotactic behavior of *D. magna* to the herbicide because of the increasingly positive-slope trend line based on the sum distance of the organisms. These results are likely due to the acting ingredients of the insecticide and herbicide respectively: deltamethrin and 2, 4-D and the receptors found in the *D. magna.* Some studies (Xie *et al*. 2005) state that 2, 4-D contains estrogenic factors, and *D. magna* have estrogen receptors (Kashian, Dodson 2002). Therefore, one possible explanation for the *D. magna* traveling toward the herbicide would be that the *D. magna* were attracted to the chemical containing the 2, 4-D. On the other hand, other studies (Du *et al*., 2010) state that deltamethrin shows weak estrogenic factors, which give insight into one of the explanations why the *D. magna* were not attracted to the insecticide.

CONCLUSION

The hypothesis was found to be true, as the organisms reacted chemotactically to the addition of chemical compounds in the lake model. *D. magna* responded with negative chemotactic properties to the addition of the insecticide, while they responded with positive chemotactic properties to the addition of the herbicide in the Lake Model. Though two different amounts of chemical compounds added respectively, no dependency of chemotactic strength in response to various volumes of chemical was found according to the quantity of added chemical compounds. This study also validated the Lake Model as a competent representative model for a lake. Although *D. magna* are not ideal models for human physiological responses, these data make it clear that chemicals in the environment have an impact. Over a much higher concentration range and period, similar results may occur in humans. For further research, the study should be continued for additional chemical compounds, while adding other variables such as current water intensity, changes in temperature, and changes in pH. To determine which ingredients of the particular chemicals are affecting the *D. magna*, the chemotaxis of *D. magna* to the individual active ingredients of each chemical might be determined. For example, rather than using the insecticide and herbicide, the main active ingredients in the different compounds might be further studied, like the deltamethrin and the 2, 4-D.

ACKNOWLEDGES

I would like to thank my mentor, Noory Shin for his dedicating time and effort into helping us complete the study.

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