

## Heartbeat and Vertical Migration Effects of Magna Daphnia by Environmental Hormones

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### Abstract

Environmental hormones are harmful synthetic chemicals that can prevent the body from functioning properly by acting as hormones. Therefore, their continual accumulation of environmental hormones can trigger many catastrophic effects to our ecological systems inconspicuously hidden from us. *Daphnia* is an important aquatic organism as a first consumer that lives in lakes and rivers. Thus, understanding the heartbeat effects of environmental hormones on the cardiac function and phototactic migration rate of *daphnia* should be of significance in an ecotoxicological sense. Among many studies, the relationship of heartbeat change and migration rate by the chemical compounds has not been much investigated.

In this study, the changes in *daphnia*'s heartbeat after 30-minute incubation was attempted to correlate with its change in migration rate induced by the phototaxis with the chemical compounds added. This study was to examine the relationship of heartbeat change and migration rate under the influence of the environmental hormones of biphenate, deltamethrin, and aatrex. Based on the comparison of the values at 1:1000 dilution solution, the mean migration rate change% was 74 %, 95%, 98%, while the heartbeat change % was 2.3%, 4.0%, 8.0%, respectively. Considering the linear relations, the migration rate was suggested to be more sensitive parameter than the heartbeat change for the three environmental hormones examined here.

**Keywords:** Environmental hormones, Endocrine disruptors, Vertical migration rate, Acute cardiac effect, Phototactic property,

### Introduction

A healthy human body is profoundly dependent on a well-functioning endocrine system for regulating the secretion of certain hormones that are essential for bodily

functions including cellular metabolism, growth, and reproduction (1,2). However, there are certain chemicals, man-made and mostly present in nature, that interfere with the normal functioning of the body's hormonal system, thus increasing susceptibility to adverse health defects (1,3). Such noxious substances are called environmental hormones, otherwise scientifically known as endocrine disruptors (1). Environmental exposure to compounds that can mimic or block the activities of hormones can trigger the severe alterations in the body's innate regulatory mechanisms and thus, may cause adverse effects in the development and immune activity of the organism (7). By simulating naturally occurring hormones in the body, the environmental hormones modify the biochemical signals that hormones carry throughout vital tissues and organs (1,7). The last two decades have witnessed growing scientific concerns and public debate over the potential adverse effects that may result from exposure to such chemicals, thus substantiating the significance of our study of the of behavioral effects that such compounds impose on wildlife.

Endocrine disruptors are most commonly found in pesticides, electronics, personal care products, additives or contaminants in food products (9). They are present in various everyday products such as plastic bottles, detergents, metal food containers, and toys, which is why a vast number of people are greatly exposed and susceptible to such harmful chemicals in their everyday lives. UN Under-Secretary-General and UNEP Executive Director Achim Steiner claimed, "Chemical products are increasingly part of modern life and support many national economies, but the unsound management of chemicals challenges the achievement of key development goals, and sustainable development for all" (11). Thus, the recent increase in the presence of environmental hormones in commonly used products raise deep concerns and the necessity to study the effects of bodily functions and behaviors as a result of exposure to these substances. Thus, this study was designed to explore the relationship of heartbeat change and vertical movement in multiple environmental hormones in *Daphnia Magna*.

The environmental hormones can be classified as follows; first, polychlorinated compounds, which are found from industrial production or by-products of widely banned substances such as polychlorinated dioxins and polychlorinated biphenyls. Second are organochlorine pesticides, found in many insecticides such as DDT, dieldrin, and lindane, from agricultural runoff and atmospheric transport. The next category of hormones are organotins, found in antifoulants used mainly for painting purposes, which include substances such as tributyltin (8). In this study, three environmental hormones: aatrex, biphenate and decamethrin were chosen to study. These chemicals have been mainly used as insecticides and pesticides in order to disclose the detrimental impact of these widely used products on aquatic organisms.

The population growth seen over the last century has resulted in an increasing demand for agricultural products to maximize crop production, thus leading the parallel growth of multiple environmental hormones, including aatrex, biphenate and decamethrin in order to maximize the crop production. One overarching repercussion for such rapid growth of these hormonal disruptors is that the exponentially growing usage of pesticides poses a great detrimental impact on many non-target aquatic organisms and their ecosystems throughout the world (3).

First of all, biphenate was chosen for observing the photo and chemotactic properties of the daphnia sample because of its characteristic as a highly effective insecticide and acaricide against a wide range of insects, as well as being very poisonous to aquatic mammals. Acute toxicity tests were performed to study the effects of biphenate on the survival, growth, and reproduction of *Daphnia* for a study of pesticide transfer from generation to generation(12).

Decamethrin is a toxic pyrethroid, a man-made version of pyrethrins insecticide toxic to many aquatic organisms, and was used to observe the behavioral effects that the compound imposes on *Daphnia Magna*. *Daphnia* and other crustaceans are more closely relate to insects than vertebrates, therefore highly selective insecticides like decamethrin may pose a greater hazard to arthropods than to vertebrates (12, 13). Decamethrin might act as an endocrine disruptor in *D. manga* as it interacts with sex determination and development abnormality.

Aatrex is an herbicide of the triazine class. Aatrex is used to prevent pre-and postemergence broad-leaf weeds in crops such asmaize and sugarcane and on turf grass used on golf courses and residential lawns (21, 22). It is one of the most widely used herbicides in the US and Australian agriculture. It was banned in the European Union in 2004 when the EU found groundwater levels exceeding the limits set by regulators, and Syngenta could neither show that this could be prevented nor that these levels were safe.

As of 2001, aatrex was the most commonly detected pesticide contaminating drinking water in the United States.

In the planktonic crustaceans, *Daphnia*, the single, small heart is easily visible when viewed under transmitted light under a low power microscope (15, 16). The heart rate can be up to 300 beats per minute and it is usually monitored and counted in various conditions such as differences in water temperature, or changing the type and concentration of chemicals dissolved in the water (25, 26). A change in *Daphnia* heart rate may serve as a basis for a predictor of a similar change in vertebrate, especially human, heart rate under similar conditions (30). Furthermore, the procedure also provides an interesting technique for investigating the effects of different chemicals on an organism's metabolism and behavioral patterns (14, 26). The heartbeat change of daphnia has been used a universal method of evaluating the toxic effect of the chemical compounds described above (27, 28, 29). However, it has been pointed out that the parameters might not be easy to verify because of intro-and inter-variability and lack of criteria for comparison of data among many other laboratories (27). In spite of such as high variability, its importance has been recognized decades ago, considering the fact that the daphnia heartbeat monitoring method has been prescribed by OECD guidelines for global standardization. In contrast to the popular study of heartbeat measurement, the chance of vertical migration speed has not been much studied. In this study, the relationship and sensitivity of heartbeat and migration rate were elucidated in more detailed way.

Part of the behavioral repertoire of *Daphnia* that is central to vertical migration is phototaxis. Understanding the *Daphnia's* behavior in response to different combinations of wavelength and light intensity is key to further understand how light affects its behavior in nature (14, 15, 23). The response to light is measured by

observing phototactic behavior. Phototaxis is the ability of organisms to physically move directionally toward (positive) or away (negative) from a light source (15). Such light cues could have a large impact on the migration patterns, circadian rhythms, and reproduction successes of these crustaceans (14, 23), which in turn impact the organisms that the crustaceans interact within lakes and ponds. In addition, the phototactic behavior of the daphnia gathered could be used as a reference to study potential toxic effects of the exposure to different chemicals (14, 24). Though the main parameters in this study have been studied separately, their relation has not been examined.

## **2. Materials and methods**

### ***2.1 Materials and Reagents***

Daphnia Magna was purchased from Carolina Biological Supplies (Burlington, NC), and maintained in a 10-gallon fish tank until tested, while being fed with active yeast mixed with flakes of fish food (25 °C, 60% relative humidity) under the 12/12 light/dark cycle.

Other instruments and materials included the Biological Science Student model compound microscope (Irvine, CA), clear acrylic sheet (OPTIX 48"x99"x0.093", Paramus, NJ), LED light bulbs (Jameco Electronics, CA), three-way switch (Radio Shack, NJ), nutrient water, silicon glue (Devcon, NJ), Rust-Oleum Semi-Gloss Protective Enamel, Black (CA). Decamethrin (Raid Max Bug Barrier, Jansen Distributing, WA), and Biphenate (Talstar P Professional Insecticide, 7.9%, FMC Corp, Philadelphia, PA) were purchased from Home Depot (Paramus, NJ). Aatrex (St. Augustine Weed Killer, 4%) was purchased from ePest Solutions (Humble, Tx).

### ***2.2 Heartbeat Counting Method***

Most other laboratories count daphnia heartbeat for 10 seconds and multiply 6 for converting its unit into beats per minute (bpm). This method was prone to error since the scientist should count its heartbeat with one eye while looking at the time on the stopwatch with the other eye. In our method, the time was measured to take 40 counts, while tapping on the table with the middle finger synchronizing with the heartbeat. In this case, the scientist didn't need to see the stopwatch and so paid attention solely to count its heartbeat of forty counts, which was found to be more accurate in our preliminary study. After the time of 40 heartbeats was acquired, its values were typed into the computer using MS Excel worksheet which calculated the heartbeat to be the unit of bpm.

### ***2.3 Preparation of Serially Diluted Solution:***

The experimental materials were collected on the table including a tube rack, six test tubes, permanent markers, the testing chemicals, a 1000 uL Eppendorf pipette and pipette tips. The six test tubes were labeled with a permanent marker from  $10^1$  to  $10^6$ . Using the pipette, 1800 uL of nutrient water was dispensed to every of the six test tubes. Then, 200 uL of the testing chemical was obtained from the bottle with original strength and brought into the first test tube. This solution was thoroughly mixed to

create a 1:10 dilution solution. Then, 200  $\mu$ L of the solution in the first test tube was taken and moved to the next test tube labeled as  $10^2$ . The solution was thoroughly shaken and mixed. This process was repeated up to the test tube labeled as  $10^6$ .

#### ***2.4 Thirty-Minute Incubation Study in the Diluted Solution***

Immediately after the incubation solution was prepared, one daphnia was taken out from the daphnia container. Its heartbeats were counted and recorded as a pre-incubation heartbeat. The daphnia was released into the solution of the first test tube and the incubation stopwatch was started to track the incubation time. Then, it was released into the solution of the following test tubes at every five-minute interval. For the second test tube, another daphnia was picked from the container at three minutes and measured the time of forty heartbeats. Then, it was released into the second test tube at 5 minutes. Likewise, with the identical methods, a pre-heartbeat counted daphnia was released into the test tube up to the test tube labeled as  $10^6$ . The daphnia for the last test tube was released at 25 minutes with the incubation stopwatch. When the incubation time arrived at 30 minutes, the daphnia incubated in the test tube  $10^1$  was taken out, and its heartbeat was counted and recorded. At 35 minutes on the incubation stopwatch, the second daphnia was taken out and its heartbeat was counted. At every five minutes of the post-incubation period, one daphnia from the following test tube was taken out and its heartbeat was counted. The process continued to the test tube  $10^6$ . Thereafter, the data was typed into the computer; and heart rate as bpm and its differences before and after incubation were calculated.

#### ***2.5 Vertical Migration Measurement***

A migration chamber was created with plexiglass plastic panel. Its dimension in length, width and height were 12 inches, 8 inches, and 24 inches respectively, and spray-painted in black to maximize the phototactic effects in the chamber. The fourth side of the chamber was removable and one-inch vertical stripe of the non-painted line was created in the middle from the bottom to the top, which made it possible to observe the movement of the daphnia through. The migration chamber was installed with a three-way switch with which two light bulbs, one at the bottom, and another at the top could be easily turned on or off alternatively. A transparent circular cylinder (3 inches I. D., 22 inches high) was created with graduated circular marking lines drawn at every inch from the bottom of the tube. The nutrient water of 700 mL could be filled in the cylinder and closed carefully after five daphnias were placed inside. The cylinder was closed with caps at both ends and no leakage of water should be ensured. The vertical migration movement was initiated by the light bulb and mostly swam toward the light. And, the marked values from the bottom were recorded at every 30 seconds for 3 minutes after their migration locations were confirmed.

#### ***2.6 Methods for Simultaneous Incubation***

Incubation study and Vertical photo reactive migration study were conducted as a set using each of the different chemicals that were used. For carrying out the study under identical conditions in heartbeat and migration rate changes, 11 daphnias were chosen into a collecting container, among which 6 were used for heartbeat change in the

serially diluted solutions, while the other 5 daphnias were placed into the migration column for migration rate measurement. The incubation study performed serially diluting the individual chemicals in 1:10 dilutions and counting the pre and post heartbeats of 6 daphnias in the diluted solutions. The post-daphnia heartbeats were counted after an individual incubation period of 30minutes.

### **2.7 Data Analysis**

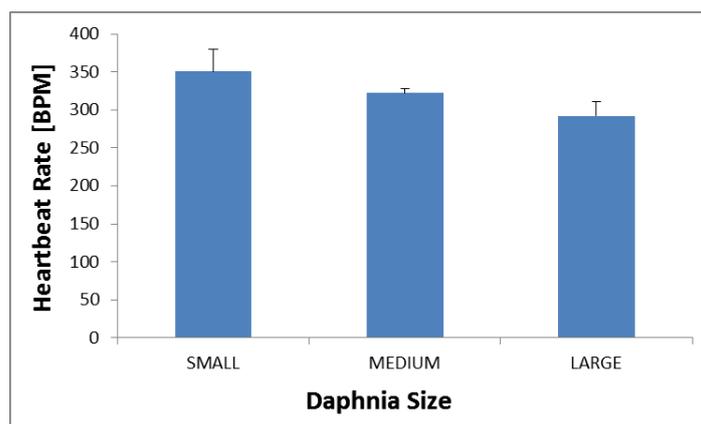
Data was presented mean and standard deviation. Student's t-test was carried out when needed ( $p < 0.05$ ). For the heartbeat measurement, after a set of study, the data was typed into the MS Excel in a PC computer, in which the heartbeat difference was calculated. The heartbeat difference was plotted with respect to the serial dilution factor. And, the relation of heartbeat difference and dilution factor was evaluated using the trend line function of the linearity. The slope of the linear line (bpm difference/dilution factor) was defined as an acute effect of the testing chemical. Set of 10 trials were done if otherwise described. For the migration rate, after filling the migration cylinder with 700 mL of nutrient water, 5 daphnias was dropped and capped securely. Before the first reading, the bottom light was on to make the daphnia move down most, then their locations were recorded such as 0, 0, 0, 1, 1. And, then the top light was turned on at 0 seconds, and their location was read and recorded at every 5 seconds. The number was averaged and plotted on the graph on time. The slope (distance/time) as a migration rate was calculated by the computer.

## **3. Results and discussion**

### **3.1. Selection of Daphnia population**

The study was started from the determination of the optimal size of the daphnia in the study. The experimental daphnia population was divided into three groups; small, medium and large after measured their sizes under the microscope with an object lens with metric scales. And, subsequently, their heartbeat was measured and recorded accordingly. Fig. 1 presented the heartbeat rate according to the size of the study population. As seen in Fig. 1, the heartbeat rate of the medium size daphnia was relatively more reproducible and manageable to care and control compared to those from other sizes.

When the daphnia's heartbeat was measured for 30-minute incubation, a data table was created in the worksheet of MS Excel as given below in Table.1. The second column was the time points as reminders when each daphnia was dropped into the test tubes filled with the serially diluted solution. The third column presented the time to take for 40 heartbeats using a stopwatch as a pre-incubation heartbeat. The fifth column was filled with the time of incubation after the first daphnia was dropped. Since it was 30 minutes in the uppermost data cell of the fourth column, the daphnia at the first test tube was picked up and counted for the heartbeat of post-incubation. The 4th, 7th, and 8th columns were automatically calculated in the sheet as a unit conversion function to be BPM and its difference.



**Figure 1:** Heartbeat rate was significantly different with respect to the daphnia size ( $P < 0.05$ ). The small size was  $< 1.0$  mm, medium, ranging from 1.0 to 2.0 mm, while the large size was greater than 2.0 mm ( $N = 20$ ).

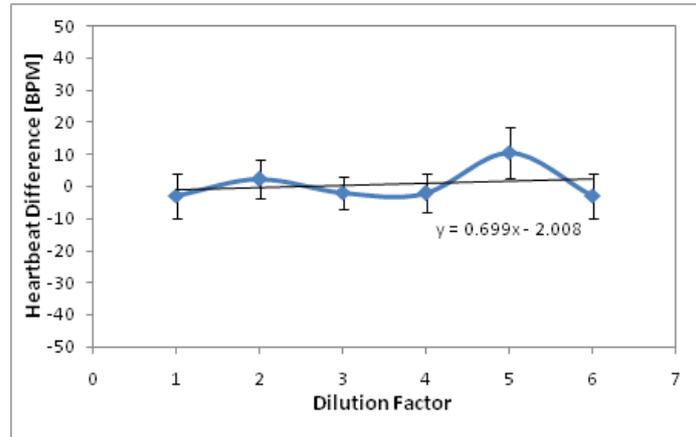
**Table 1:** The tabulated form for summarizing raw data for converting to bpm unit and plotting

1	2	3	4	5	6	7	8
Dilution Factor	Pre-Incu t (min)	t/40 cts (sec)	bpm	Post-Incu t (min)	t/40 cts (sec)	bpm	bpm difference
$10^1$	0	8.60	279	30	9.00	276	-3
$10^2$	5	8.19	293	35	9.55	295	2
$10^3$	10	8.41	285	40	9.59	283	-2
$10^4$	15	8.13	295	45	9.81	293	-2
$10^5$	20	8.69	276	50	9.00	287	11
$10^6$	25	8.44	284	55	9.83	281	-3

The following graph was plotted on the BPM difference with respect to the dilution factors. The slope, heartbeat difference divided by the dilution factor was defined cardiac toxicity. When the slope was less than 3.0, the chemical could be defined to have a minimal cardiac effect, while any slope larger than 3.0 was considered to have a toxic effect to the heartbeat of the testing organism.

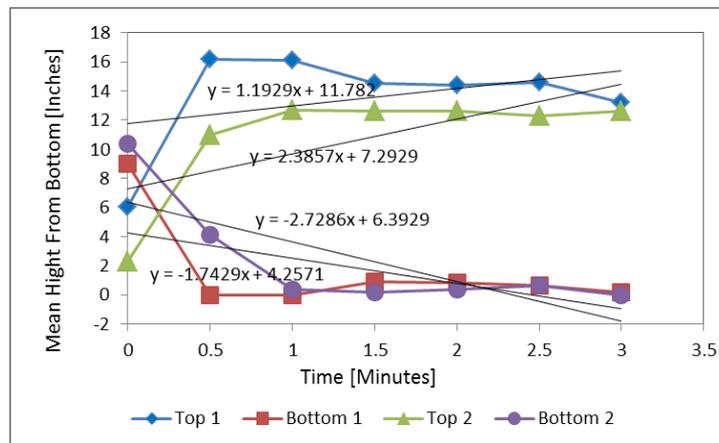
### 3.2 Negative Control in Heartbeat and Migration Rate

For a negative control, the daphnia was subjected to the 30-minute incubation study in the nutrient water which is the culturing water obtained from the daphnia tank. As seen in Fig. 2, as expected, only minor change has been recorded with normal standard deviation, which could cause the organisms' inter-variability and experimental conditions. In this negative control study, the slope of the heartbeat change according to the dilution factor was found to be 0.699. So, the minimal cardiac effect by the incubation in the culturing water was recorded as no acute cardiac effect as one might be expected.



**Figure 2:** The change of heartbeat after 30-minute incubation in culturing water only. No significant change of heartbeat was found in any set of study within the variability usually experienced under normal condition.

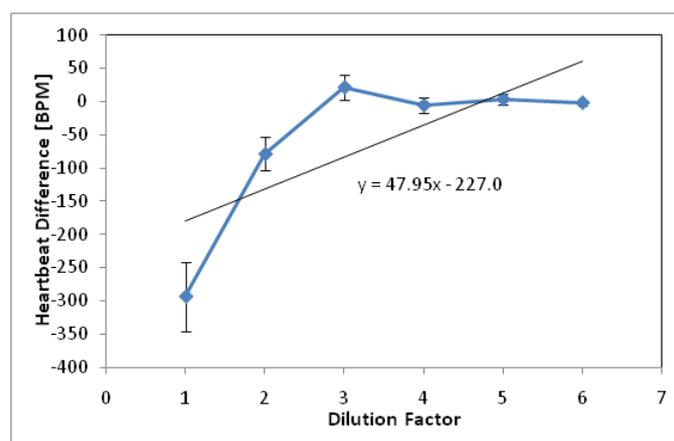
On the other hand, Fig.3 illustrated the typical graph of height change of daphnia in the column while being incubated in the culturing water with respect to the time by the induced phototaxis. As described in the method section, each daphnia’s location was recorded at every 30 seconds for 3 minutes and then, the alternative light was powered from bottom to top and from top to bottom for four times. The slope, distance divided by time was calculated using the trendline function. As seen in the figure, the mean absolute slope was estimated to be 2.0, which was a typical magnitude of swimming rate when measured before the addition of any chemical solutions.



**Figure 3:** A typical movement of daphnia when incubated only in the culturing water. The mean of five daphnia location at every 30 seconds for 3 minutes. Top 1 meant light on top bulb first, bottom 1 light down bulb on, top2 meant a second time of top light on and bottom 2 was the bottom light on alternatively.

### 3.3 Positive Control in Heartbeat

For the positive control, the 70% ethyl alcohol was used to see the change of heartbeat after 30 min incubation in serially diluted solution as seen in Fig. 4. As evaluated in contrast to our negative control, the slope of heartbeat-dilution plot was 47.96, which was significantly higher than that of the negative control. Any chemical compounds that might disturb the heartbeat could initiate other physiological effects in the end. The data was expected to have a high change in its heartbeat mostly agreed with other studies (31).



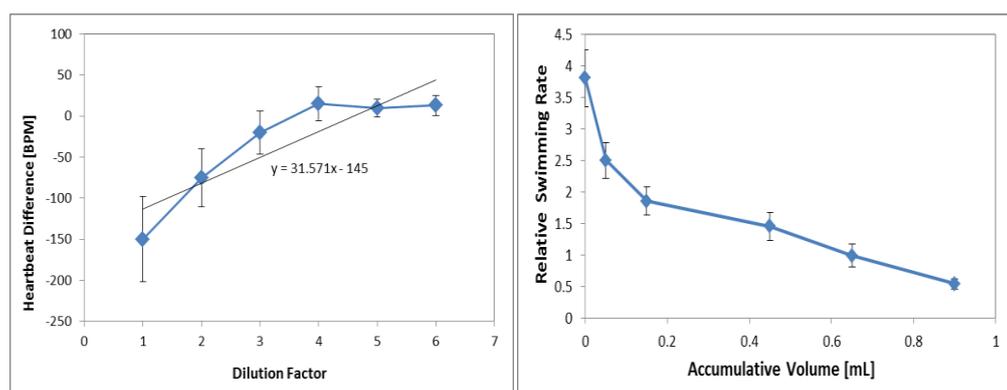
**Figure 4:** The heartbeat change with respect to the diluted solution with 70% ethyl alcohol. The general trend of heartbeat change has been agreed with other published articles.

### 3.4 Cardiac and Phototactic Effects by Biphenate

The slope of the linear fitting curve from biphenate 31.6, which was smaller than that of decamethrin as demonstrated in Fig. 5a. On this graph, the heartbeat difference under the dilution solution with  $1.0 \times 10^3$  dilution was approximately 5 bpm diff which change was estimated 5% compared to the baseline heartbeat. However, their trend of decrease with respect to the increase of the dilution factor was similar. It could mean the method might be a good indicator of a cardiac toxicity applicable for multiple chemical compounds.

These group of daphnia had a stronger affinity towards light coming from the top than when the light was switched on from the bottom as seen in Fig 5b. Biphenate has added accumulatively until all the daphnia didn't respond to the light. A typical observation could be described as below: After a no-chemical baseline measurement, 0.01 mL of biphenate added into the column and shaken slowly. Then, the phototactic test was started. Typically, the daphnia became more polarized in distance relative to the direction of the light. When the light was switched on to the top from the bottom, the majority of the daphnia traveled towards the maximum height. This result suggested that biphenate might increase daphnia's sensitivity towards the light. Then, 0.02 mL biphenate was added more into the column and observed, while recording.

At this time, the majority of the daphnia lost their vertical migration ability and their photosensitivity. When they experienced a weakening in their photoreactivity it was due to their reduction in reaching their potential in vertical migration abilities. This was evidenced by one daphnia that attempted to swim towards the light's direction, but could not because it started losing its migration ability. Daphnia tested with biphenate elicited the moderate effect in limiting their migration abilities compared to other chemicals of insecticides and herbicides. With the chosen amount of biphenate solution equivalent to  $1.0 \times 10^3$  dilution factor, the migration rate was changed up to 74%.

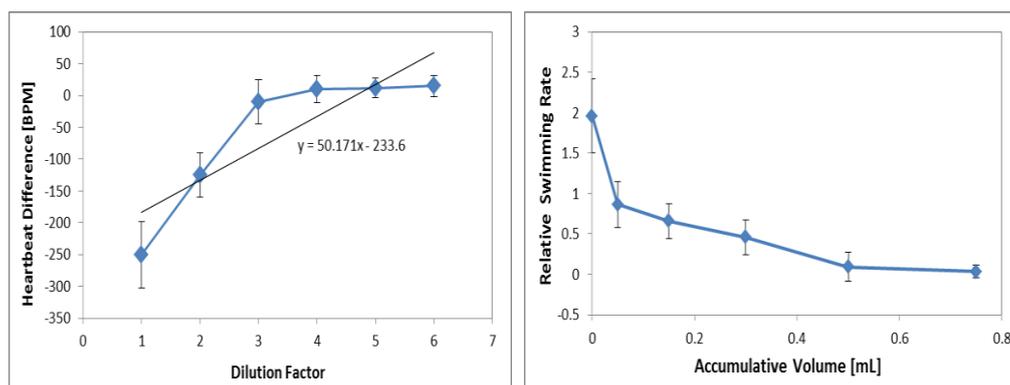


**Figure 5:** (a) The heartbeat change with respect to the dilution factor of biphenate (b) The relative migration rate according to the addition of biphenate. Dilution factor 3 at (a) is the identical solution 0.7 [mL] in (b). If compared, the migration rate changed 74% significantly more than heartbeat change 2.3% at the points indicated.

### 3.5 Cardiac and Phototactic Effects by Decamethrin

The graph shows the relation of heartbeat difference before and after the thirty-minute incubation in serially diluted decamethrin solution as seen in Fig. 6a. As expected, the heartbeat change was decreased with respect to the increase of dilution factor with a high slope of 50.17. With the chosen amount of decamethrin solution equivalent to  $1.0 \times 10^3$  dilution factor, the migration rate was changed 2.3%.

In the case of migration rate measurement, when 0.05 ml decamethrin added into the column, it became apparent two of the daphnia still had the ability to swim vertically and maintain photosensitivity. Effects of 0.05 ml decamethrin were significant because three of the daphnia had decreased vertical migration ability and detected less photosensitivity as in Fig. 6b. Three of the daphnia lost their ability to swim vertically at 0.1 ml of decamethrin. Results were significant because two of the daphnia that was able to swim vertically lost their photosensitivity. This was evidenced by one of the daphnias that disregarded the direction of the light despite being able to swim substantially upwards.



**Figure 6:** (a) The heartbeat change with respect to the dilution factor of decamethrin (b) The relative migration rate according to the addition of decamethrin. Dilution factor 3 at (a) is the identical solution 0.7 [mL] in (b). If compared, the migration rate changed 95% more than heartbeat change 4% at the points indicated.

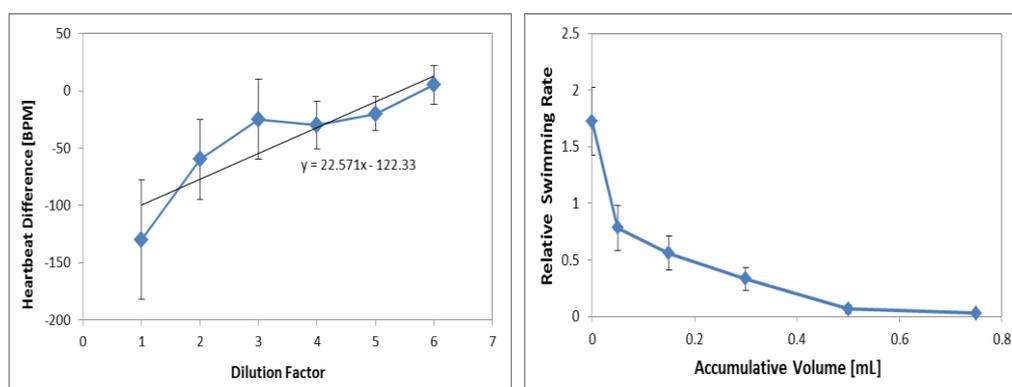
When 0.15ml added, two of the daphnia still possessed vertical migration abilities. However, there were less frequent and less substantial vertical migration movements. The two daphnia showed loss of photosensitivity as evidenced by the daphnia migration in opposite direction of the light's source. Thus, the conclusion could be made that photosensitivity with the influence of the chemical decamethrin was lost prior to the loss of vertical migration ability. Further, all four daphnias lost their vertical migration ability, when 0.2 ml was added; consequently, their photosensitivity was lost. One of the daphnias maintained its vertical migration ability. However, its ability to detect photosensitivity was inconsequential due to occasionally traveling polar opposite of the light's source. During the acclimation period where the light was switched on, the daphnia with vertical migration ability favored remaining at the container's base. In other words, when the light was switched off to condition the daphnia at a neutral state in terms of light, it almost always traveled towards 0 inches. Then, when 0.25ml added, all the daphnia lost their vertical migration abilities. They were; however, mobile horizontally. Thus, presence or the absence of light was inconsequential to the phototactic reactions of the daphnia. The migration change% to 0.7 mL equivalents of  $1 \times 10^3$  as the dilution factor and the migration rate was changed 95% at the accumulative volume.

### 3.7 Cardiac and Phototactic Effects by Aatrex

Fig. 7a shows the heartbeat change with respect to the dilution factor. The heartbeat change rate was 22.57. The slope was relatively lower than that from the other compounds.

For the phototactic effect from the aatrex addition, these group of daphnia was highly responsive to the lights'source. They almost always reacted immediately by traveling towards the light when it was switched on as seen in Fig. 7b. They also had competitive migration rates as shown by the distance they traveled in relation to time. When 0.05ml added, inconsistencies started to develop in the daphnia's

photosensitivity pattern. For example, some of the daphnias traveled in the opposite direction of light. These results might signify as initial markers for photosensitivity weakening. There was no significant reduction in vertical migration abilities.



**Figure 7:** (a) The heartbeat change with respect to the dilution factor of aatrex (b) The relative migration rate according to the addition of aatrex. Dilution factor 3 at (a) is the identical solution 0.7 [mL] in (b). If compared, the migration rate changed 98% more than heartbeat change 8% at the points indicated.

Adding the volume ranging from 0.1ml to 0.2 ml, inconsistencies continued to develop in the daphnia's photosensitivity pattern. For example, some of the daphnias traveled in the unpredictable direction of light with no clear pattern. There were initial signs of lethargy in the vertical migration abilities in few of the daphnia. However, these signs were not significant. When 0.25ml added, incoherent behaviors continued to develop in the daphnia's photosensitivity pattern.

When 0.3ml added, inconsistencies continued more outstandingly in the daphnia's photosensitivity pattern. Some of the daphnias traveled in the opposite direction of light. These results may signify as initial indicators for photosensitivity weakening. There were initial signs of lethargy in the vertical migration abilities in few of the daphnia. When 0.35ml to 0.4 ml aatrex added, a behavioral disarray continued to increase in the daphnia's migration pattern with more serious suppression of photosensitivity. When 0.7 mL added accumulatively, the percent change of the migration rate was down to 94%.

### 3.8 Direct comparison of all three compounds

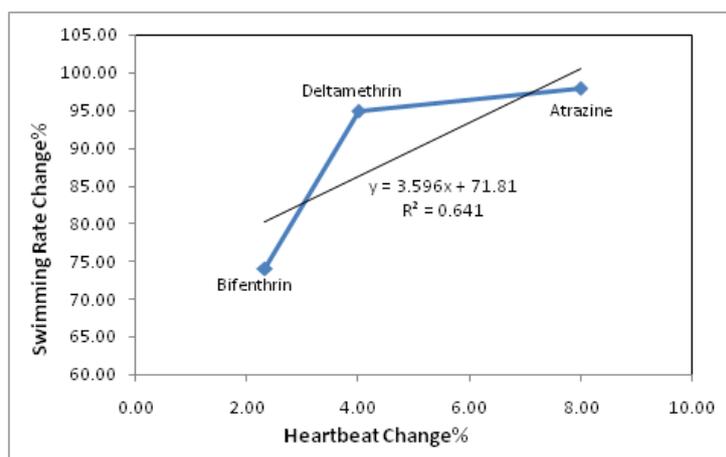
Based on the comparison of the values at  $1 : 10^3$  dilution solution, the percent change of migration rate in phototactic property was 74, 95, 98 % in the dilution solution of biphenate, decamethrin and aatrex, while the heartbeat change % was 2.33%, 4.00%, and 8.00%, respectively. The migration rate was significantly changed more than the heartbeat change in all the three environmental hormones.

Daphnia that was tested with biphenate had the strongest effect in limiting their migration abilities compared to other chemicals of insecticides and herbicides. Because previously tested daphnia using Biphenate lost their migration ability at a

much lower biphenate dosage, the second trial to test daphnia photosensitivity was done using a lower Biphenate volume compared to the other chemicals daphnia were tested with. When decamethrin was tested on daphnia, the initial hypothesis was also not substantiated because the daphnia's photoreactivity was lost simultaneously with their vertical migration ability during the lower chemical incubation

Decamethrin was similar to biphenate in the sense it was high in toxicity as evidenced by a rapid loss of vertical migration ability in the majority of the daphnia. All five daphnia had lost their vertical migration abilities during both the Biphenate and decamethrin incubations.

Compared to decamethrin, aatrex had the least effect on daphnia's vertical migration motions. The study conducted using aatrex consumed the most time because discontinuation of successive chemical addition was determined based upon the loss of vertical migration ability in the majority of the daphnia. When the vertical migration motions were not lost like in the case of aatrex addition, the point of study cessation was determined by the loss of photosensitivity in the majority of the daphnia. Aatrex chemical affected photosensitivity at the lowest chemical dosage while affecting vertical migration ability minimally with the highest chemical concentrations. As demonstrated in Fig. 8, there existed a linear relation of parameters between the heartbeat change and migration rate.



**Figure 8:** The relation of the relative migration rate and heartbeat change was relatively linear ( $R^2=0.64$ ). In all cases, the change% of the relative migration rate was significantly greater than the heartbeat change.

### Conclusion

The environmental hormone's effects on heartbeat and migration were investigated. In conclusion, it suggested that the migration rate induced from the phototactic property was more sensitive than the heartbeat change for 30-minute incubation study. Based on the comparison of the values at 1 :1000 dilution solution, the percent change of migration rate in phototactic property was 74, 95, 98 % in the dilution solution of biphenate, decamethrin and aatrex, while the heartbeat change % was 2.33%, 4.00%,

and 8.00%, respectively. The relation of the heartbeat change% with the migration rate% was relatively linear. More study might be needed for a diverse variety of environmental hormones.

### Acknowledgements

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