Quantifying the phototactic response of *Daphnia magna* at the laboratory scale

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Abstract

Zooplankton are only a few millimeters wide but travel several meters daily. Diel vertical migration (DVM) is the diurnal movement of zooplankton from a waterbody's surface at night, to a depth during the day. This movement is a phototactic response to sunlight. The motions of DVM may affect the surrounding environment by inducing biomixing and affecting the nutrient content, upwelling regimes, and local stratification as a result. However, this escape from light (negative phototaxis) only occurs in the wild, in the laboratory they swim towards it (positive phototaxis). This change in behavior is heavily related to light conditions, however the reasoning for it remains unexplained. The research described in this paper uses laboratory scale experiments to investigate the effects of light weather on the phototactic response of a common zooplankton species, *Daphnia magna*. The research aims to further the understanding of phototaxis in *D. magna* and how their phototactic response can be replicable in a laboratory environment. Using a tank with an LED light set-up to drive upwards and downwards phototaxis, four wavelengths (red, green, blue, and white) and four light intensities (100%, 75%, 50%, and 25%) were investigated. Particle tracking velocimetry methods (PTV) were used to track the motions of individual *Daphnia magna* through a camera and extract that data for differing light conditions. The data extracted provides insight into what drives positive and negative phototaxis. Light intensity has little to no effect on the strength of the phototactic reaction, however wavelength does. Green and red light produce strong positive phototaxis, commonly observed in other laboratory experiments, while blue light produces strong negative phototaxis, like that observed in the wild. With this greater understanding of the phototactic response driving DVM, the role of DVM on macroscale conditions can now be further explored through more comprehensive laboratory experiments that are increasingly applicable to field observations.

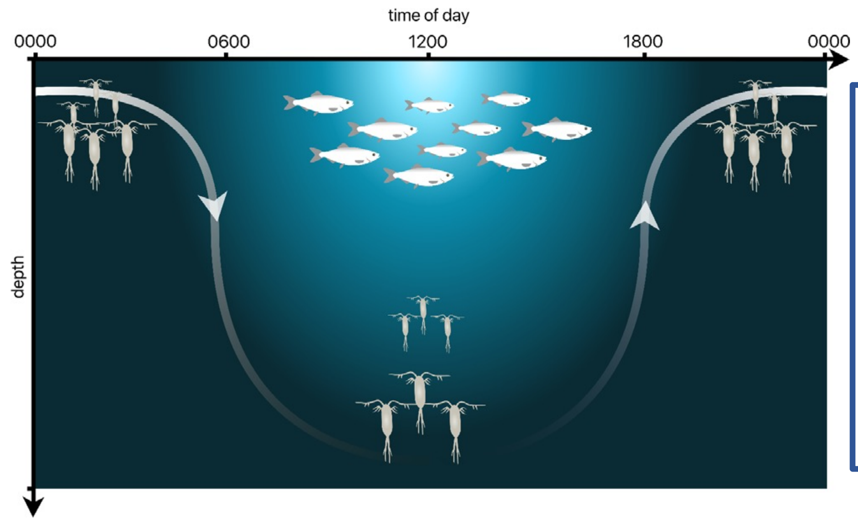

Figure 1: Diagram displaying DVM as a predation avoidance tactic, the zooplankton hiding at depth from predators (fish) at the surface during the day and returning to the surface to feed at night. (Bandara et. Al, 2012)

Methods:

The laboratory set-up for the experiment consists of a 40 by 12 by 12cm acrylic tank mounted on an aluminum frame. Attached to the frame above and below the tank is a light diffuser, attached to the diffuser and facing the tank is an LED board. The LED board is then connected to a dimmer that controls light intensity and an Arduino robot that is coded to control when each LED turns on and off. A mirror is placed diagonally through the tank in order to allow for 3-dimensional position data to be obtained.

Two colonies of around 200 *Daphnia magna* were raised from an initial colony of about 30. The original colony was purchased from Carolina Biologica. The *D. magna*'s food source was a mixture of equal parts spirulina powder, baker's yeast and chickpea protein. Their water was adjusted biweekly, the water is re-mineralized water created from deionized water with added minerals from the SeaChem solution.

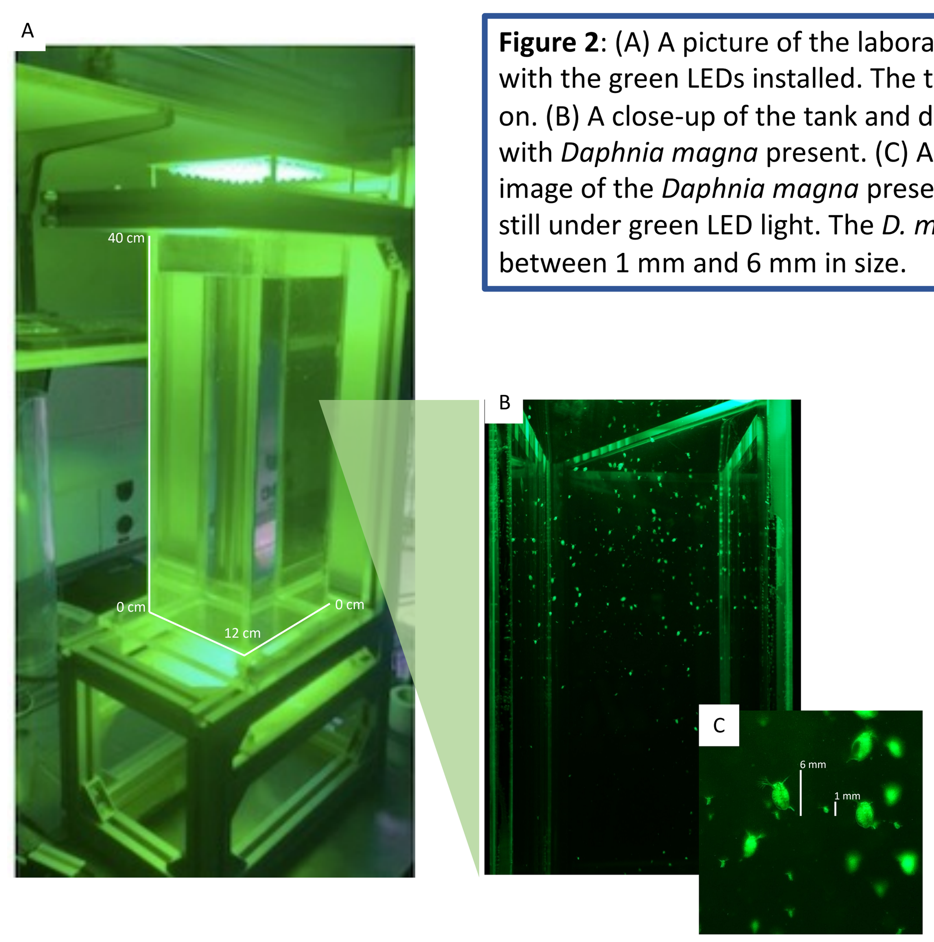

Figure 2: (A) A picture of the laboratory set-up used with the green LEDs installed. The top LED is turned on. (B) A close-up of the tank and diagonal mirror with *Daphnia magna* present. (C) A very close-up image of the *Daphnia magna* present in the tank, still under green LED light. The *D. magna* are between 1 mm and 6 mm in size.

Methods:

The laboratory set-up of the experiment investigated 4 wavelengths (red, green, blue, and white) at 4 light intensities (25%, 50%, 75% and 100%). For each experiment, about 100 *D. magna* were added to the tank filled with re-mineralized water 24 hours prior. The water level was then adjusted to 360 mm before the experiment to have equal distance between the water and the top and bottom LEDs. Each of the 16 experiments had three 10-minute trials that were ran consecutively. Each trial consisted of a 5-minute period with the top LED on, immediately followed by a 5-minute period with the bottom LED on. The timing of these trials were controlled by the Arduino robot and the light intensity was manually adjusted by the dimmer prior to the experiment. The motion of the *D. magna* was captured by a camera perpendicular to the tank. The images from the camera were than processed in order to create a tracking mechanism for the motion of the individual *D. magna* through the course of the experiment. A 3D trajectory was obtained by using the distance between a *D. magna* and its reflection in the mirror diagonally through the tank to determine the depth of the *D. magna* away from the camera (y-position). The depth away from the camera combined with the height (z-position) and the horizontal position (x-position), 3D position data was obtained and measurements for size, velocity and acceleration were also calculated.

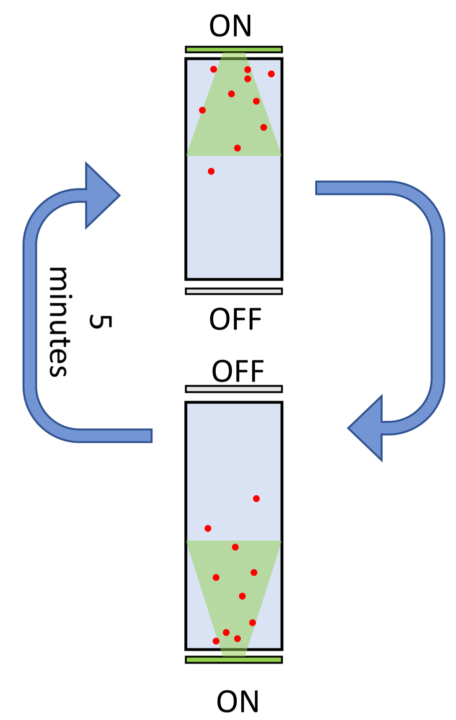

Figure 5: The basic pattern of the experiments was 5 minutes with the top LED on followed by 5 minutes with the bottom LED on, repeated for 3 trials. The red dots represent the *D. magna*.

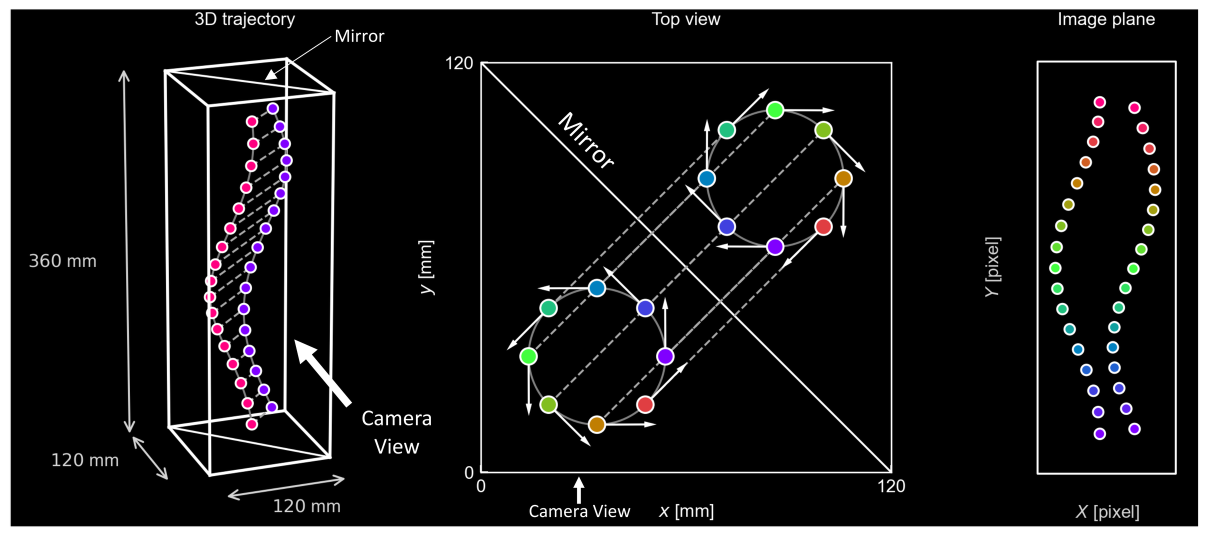

Figure 6: Methods by Dr. Daisuke Noto displaying the use of the diagonal mirror in order to determine the y-position. The distance between the *D. magna* and its reflection in the mirror was used to determine y-position within the tank (or depth from camera).

Methods:

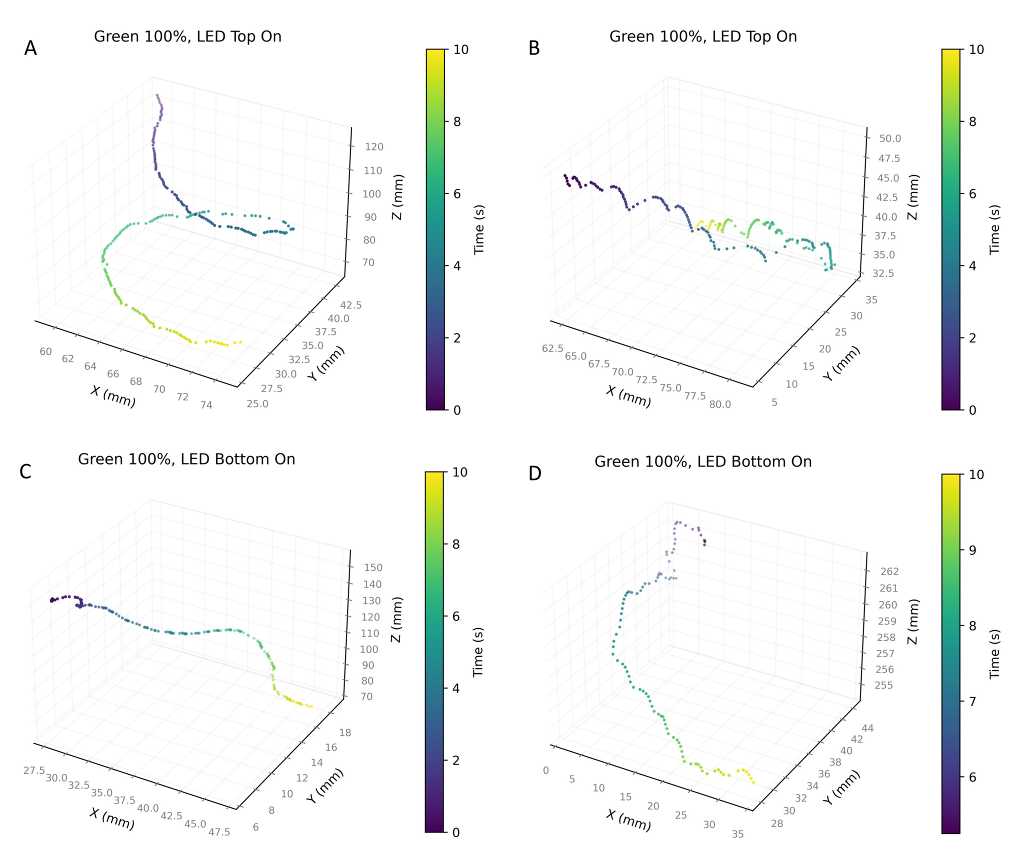

Figure 7: 3D trajectories of individual *D. magna*. Purple represents second 0 since light turned on and yellow represents second 10, therefore the trajectory starts at the purple color and ends at the yellow. All trajectories are for the green LED at 100% intensity. (A) and (B) are trajectories with the top LED on. (C) and (D) are trajectories with the bottom LED on.

The determined 3D position data allowed for 3D trajectories to be developed. The individual paths of each *D. magna* can be calculated and was observed to create an understanding of the individual motions making up the overall phototactic motion. For the green LED, a positive phototaxis occurred as the the 3D trajectory started at the bottom and moved to the top when the top LED was on, and opposite for the bottom LED being on. These trajectories confirmed the accuracy of the tracking methods used and the presence of phototactic motion. The downward trajectories were a lot more direct than the upward ones, probably the result of gravity making it easier for the *D. magna* to go down than up.

References:

Bandara K., Varpe O., Wijewardene L., Tverberg V., & Eiane K. (2021). Two hundred years of Zooplankton Vertical Migration Research. *Biological Reviews*, *96*(4), 1547–1589.

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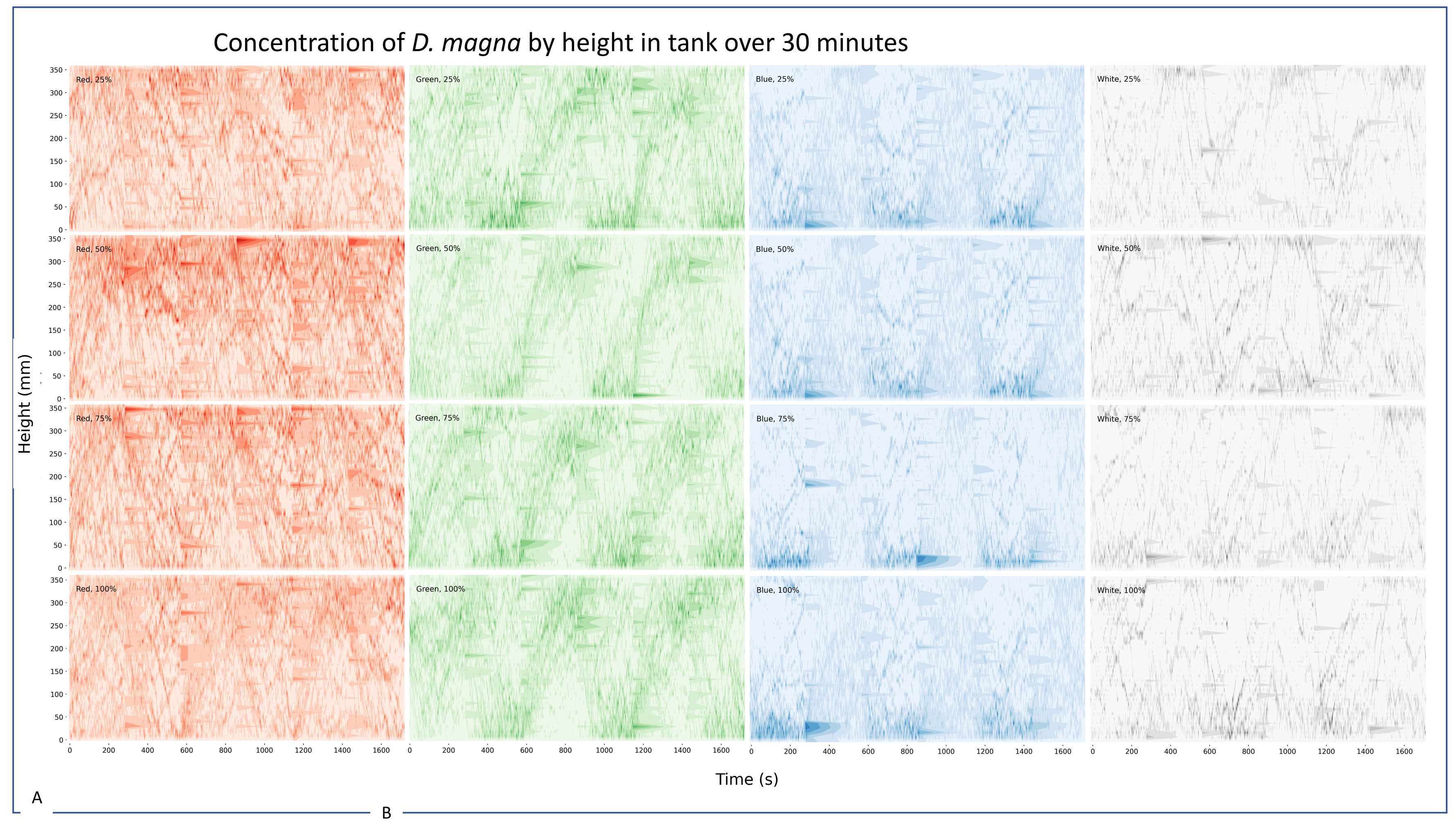
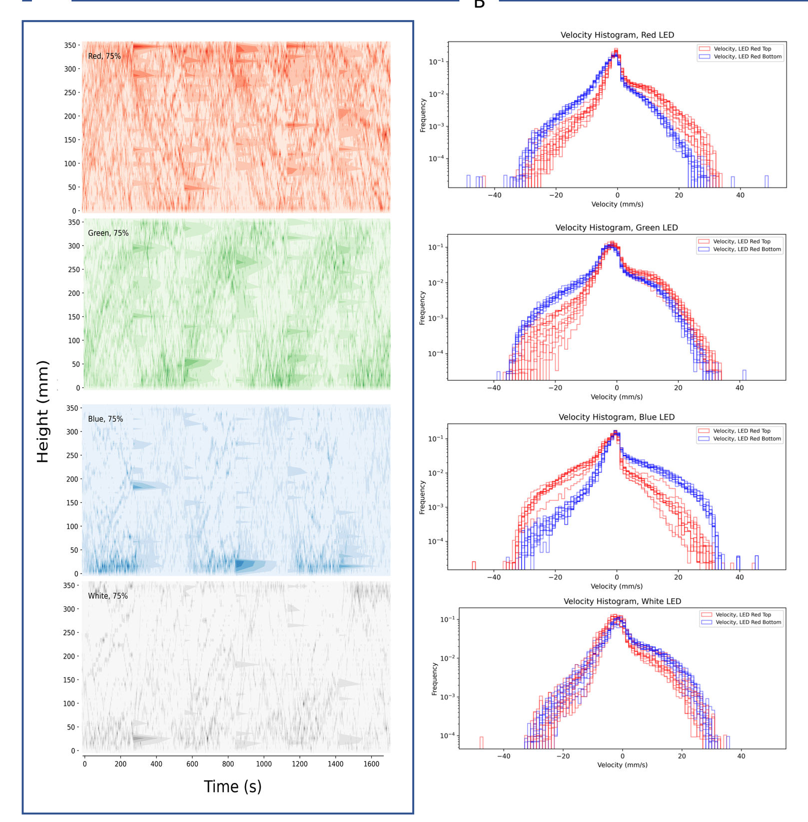
Results:

Observing the periodic reactions of the *D. magna* with respects to changes in light intensity, the differences caused by differences in light intensity is negligible. The below graphs were made over the course of the experiment and display the experimental series of the top LED being on followed by the bottom LED being on repeated 3 times. The product is wave-like functions that mimics diel vertical migration. However, looking down the columns to compare light intensity within each color, the differences are not noticeable. This suggest light intensity does not influence the strength of phototaxis, instead there may be a critical light threshold that initiates phototactic motion and phototactic motion does not change with increasing intensity past this point. From the below graphs we can determine, however, a critical period of motion in the first 30 seconds of an LED turning on. The first 30 seconds is when the greatest amount of movement occurs

Figure 3: Graphs displaying the frequency of *D. magna* at each height within the tank (y-axis) for each time series (x-axis) over the course of the experiment. The darker the color, the higher the frequency, or concentration, of *D. magna* at the height. There is a graph for each light intensity (25% at top then 50%, then 75%, then 100%) for each color (red, green, blue and white light).





Wavelength does have an affect on phototactic motion. The graphs of frequency show a clear trend of positive phototaxis from the red and green wavelengths and negative phototaxis from the blue. White light shows a trend of positive phototaxis, but it is a weaker trend than red and green. This may be the result of white light being a combination of wavelengths that includes red, green and blue. This observation was further confirmed by the velocity histograms as positive velocity shows upwards motions and is associated with the top LED being on for red and green, but with the bottom LED being on for blue. The opposite is true for the bottom LED. Again, the results for white light are noisy and not as clearly positive as red and green. All the histograms, however, have a negative shift which is the result of gravity. *D. magna* are denser than water and therefore sink when not actively moving upwards, this results in a constant negative velocity from gravity that shifts the velocity data towards the negative.

Figure 4: (A) The frequency of *D. magna* at each height over the course of a full experiment for all four colors colors at 100% intensity. (B) The histograms of velocity graphed in log scale for all four colors. The redlines represent all occurrences, no matter the intensity, of the top LED light being on and blue lines are the same for the bottom LED light being on. All velocities are from the first 30 seconds of each LED turning on, focusing on this critical period of motion within the experiment.

Conclusion and Future Research:

From these experiments, it is determined that diel vertical migration can be scaled down and readily mimicked at the laboratory scale. The phototactic motion that drives DVM allows this to happen and is heavily influenced by wavelength, but not light intensity. Light intensity does not influence the strength of phototactic motion, indicating the possibility of a critical light threshold that initiates phototactic motion. Wavelength, however, does influence phototactic motion as both red and green produce strong positive phototaxis, white produces a weaker but still positive trend of phototaxis and blue produces negative phototaxis, like the negative phototaxis that occurs in the wild. The fact that blue is closest to UV light in wavelength on the light spectrum maybe be a contributor to this relationship as a major difference between synthetic light and natural light is the presence of UV rays. While looking at this motion, a driver in its intensity is also gravity as the overall velocities of the *D. magna* had a negative bias due to the fact *D. magna* are denser than water and will sink unless they are actively moving upwards.

The suggestion of a critical light threshold and the possible influence of UV light on the direction of phototaxis are two aspects of future research to be investigated. The tank used in these experiments was too shallow to successfully replicate the degradation of light that occurs over much deeper water columns, allowing for a much more precise light intensity threshold. The ability to readily mimic DVM in the laboratory, however, provides a basis for much more comprehensive research surrounding DVM and its possible implications on the surrounding environment. The movements of zooplankton through the water column could have affects on nutrient content as the mass migration that occurs may result in biomixing. Investigating if and how this biomixing may occur could provide insight into how DVM may alleviate some of the stressors an increasingly stratified ocean will induce due to climate change.