JOURNAL OF
EMERGING INVESTIGATORS

Article

How does light affect the distribution of *Euglena sp.* and *Tetrahymena pyriformis*

Riya Singh[1], Michael Edgar[1]
[1] Milton Academy, Milton, Massachusetts

SUMMARY

The broad range of energy sources utilized by organisms to obtain organic molecules from their environment include plants, animals, and sunlight. Differences in migration patterns and anatomy can often be attributed to these differing energy sources across organisms. To evaluate the distribution patterns of organisms *Euglena sp.* (*Euglena*) and *Tetrahymena pyriformis* (*T. pyriformis*) both organisms were treated with light. *Euglena* is a genus of more than 1,000 species consisting of single-celled flagellates that primarily live in freshwater and saltwater, and *T. pyriformis* is a single-celled, ciliated eukaryote that typically inhabits fresh water. Tubes filled with either *T. pyriformis* or *Euglena* were exposed to three different treatments: entirely to light, entirely to darkness, or exposed on one half to light and the other half to darkness. Results show that *Euglena* exhibited a phototactic response to light, as there was a statistically significant 17 organism increase on the side exposed to light and an 8 organism decrease on the side exposed to darkness after the 30-minute period. *T. pyriformis* did not show any significant redistribution after exposure to any of the treatments. Due to *T. pyriformis's* heterotrophic nature and *Euglena's* autotrophic nature, these findings were most likely the result of characteristics that aid *Euglena* as autotrophs and are absent in *T. pyriformis*. Specifically, *Euglena* have a red eyespot that locates areas of light and chloroplasts. Red eyespots and chloroplasts are two of the many photosynthetic organelles that facilitate the process of photosynthesis in areas of light, making lighted areas optimal for the survival of *Euglena* in comparison to areas of darkness.

INTRODUCTION

Studying the movement of organisms is crucial to understanding the manner in which groups locate food in particular environments. Specifically, differences in the relative way organisms move can signify unique characteristics and behaviors that contribute to diverse ecosystems and patterns of migration. *Euglena sp. (Euglena*) are a single-celled flagellated eukaryotic organisms that are commonly found in saltwater and freshwater, and *Tetrahymena pyriformis (T. pyriformis*) are a unicellular eukaryote that are common in freshwater ponds (1, 2). *Euglena* are particularly unique because of their ability to switch from heterotrophy to autotrophy (1). Heterotrophic feeding means that an organism relies on external sources, such as plants or animals for their energy and nutrients, while autotrophic feeding means that an organism is able to make its own food from raw energy, such as sunlight (3). *Euglena*'s red-eyespot near the base of their flagellum allows them to move toward areas with high-light, where they will manufacture food (4). These areas of light are useful to *Euglena* as they create their own food through photosynthesis, the process of absorbing light to synthesize food. *T. pyriformis* cannot switch between methods of finding or making food, and are solely heterotrophs, or organisms that rely on the presence of external sources to absorb as an energy source and fuel its bodily processes (2). An example of a heterotroph's unresponsiveness to light can be found in the ocean. Phytoplankton only exist at the surface and are autotrophs. Zooplankton, on the other hand, can exist at all depths of the ocean due to their heterotrophic nature and consequent ability to find external food sources at varying depths (5). In the case of Phytoplankton and Zooplankton, their different ways of obtaining organic molecules gives reasoning to their drastic habitat differences. In *Euglena* and *T. pyriformis,* investigating their differing reactions to light may provide information pertaining to their distribution differences in their respective environments. A previous experiment suggested that *Euglena* respond to light with phototaxic responses, the movement of an organism in response to light either towards or away from the source, and photokinesis, a change in velocity of an organism's movement in response to light (6-8). Research has shown that the phototactic response of *Euglena* is the result of a 400 kDA photoactivated adenylyl cyclase (PAC) photoreceptor (6). Minimal research has been done in relation to *T. pyriformis* and its photostimulation. Therefore, due to the presence of a red eyespot that allows for the recognition of light and the presence of multiple PAC photoreceptor throughout their anatomy, we hypothesized that *Euglena* would have a higher distribution in areas exposed to light, while the *T. pyriformis* would remain unaffected in its distribution due to its heterotrophic nature. In this experiment, we investigated the effect of light on the distribution of *T. pyriformis* and *Euglena* and found that *Euglena* experienced a significant change in distribution towards areas of light as opposed to darkness. The finding of *Euglena's* distribution towards light will provide a foundation to study its vertical migration patterns.

RESULTS

In order to test the ability of *Euglena* and *T. pyriformis* to respond to light, we measured movement of both in response to exposure to light. An initial count of organisms before and after a 30-minute exposure to light was calculated. We performed

three replicates for each condition, and the changes reported hereafter are from the average of the three replicates' data points. Exposure to light resulted in an uneven distribution of *Euglena* across the halves of a capillary tube, while there was an even distribution of *T. pyriformis*. Exposure of *Euglena* and *T. pyriformis* tubes to darkness or light on both sides did not result in any statistically significant differences in distribution between the two halves of the capillary tube before and after the treatment (**Figures 1, 2**). Through various treatments of light - one treatment of exposure to both halves of a capillary tube to light, one treatment of exposure to both halves of a capillary tube to darkness, and one treatment of exposure of one half to light + the other to darkness, - a clear, significant change in the distribution of *Euglena* towards areas of light revealed the organism's phototactic behavior (**Figure 1**) (*t*-tests: Light + Dark Treatment: Light *p*-value = 0.0014, Dark *p*-value = 0.0004, Light + Light Treatment: *p*-value > 0.05, and Dark + Dark Treatment: *p*-value > 0.05). In tubes of *Euglena* simultaneously exposed to both light and dark, there was an increase of 17 organisms on the side exposed to light and a decrease of 8 organisms on the side exposed to darkness (**Figure 1**). There was no significant effect of exposure to any of the three treatment groups for the *T. pyriformis*, as all changes in distribution were not significantly different from each other (**Figure 2**). (*t*-test for all differences in distribution throughout trials = *p*-value > 0.05) Overall, the data suggests that *Euglena* are more drawn to areas exposed to light rather than darkness, while *T. pyriformis* do not exhibit a preference for either light or darkness.

DISCUSSION

Our results support our hypothesis and yield the conclusion that when exposed to both darkness and light, *Euglena* prefer to redistribute toward the light. Additionally, our data supports our hypothesis that neither light nor darkness affects the distribution of *T. pyriformis*.

Euglena have a red eyespot on their anterior end that allows them to detect light (4). This eyespot is used to help the *Euglena* find bright areas where sunlight is present for photosynthesis to be carried out as a mechanism of survival. Photosynthesis is the process of utilizing sunlight through the absorption of light to synthesize foods from carbon dioxide (CO_2) and water (H_2O) (9). In *Euglena,* the red eyespot allows for the organism to generate a phototactic response to its recognition of light and results in the majority of organisms reaching a suitable location where optimal conditions are met. While the red eyespot facilitates the redistribution of *Euglena*, the chloroplasts provide an incentive for *Euglena* to redistribute. Chloroplasts are the organelles that trap the sunlight once the organisms reach areas of light, initiating the process of photosynthesis and allowing for the condition of light to be optimal for the *Euglena*'s survival (1).

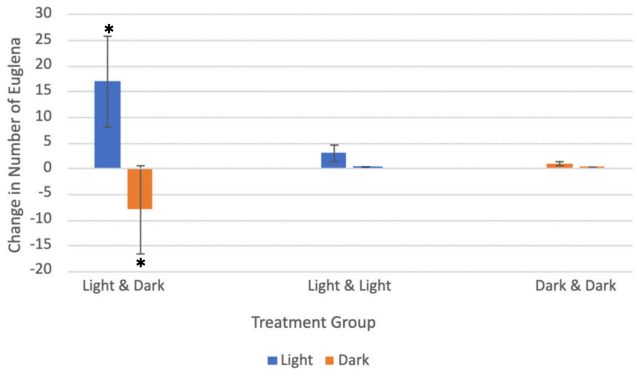

Figure 1: Exposure to light initiated a phototactic response in *Euglena*. Light exposure to two halves of a capillary tube was altered in the three treatments by a tinfoil wrapping. The tubes exposed to light on both sides and those exposed to darkness on both sides were the control groups. Tubes were left under a light source for 30 minutes. The change in the average sum of three counts of organisms on each side of the capillary tube before and after exposure to its treatment was obtained and shown. Error bars indicating AAD are shown. Bars with asterisks above them indicate that the post-treatment count was statistically different from the pre-treatment count (*t*-test: $p<0.05$, n=3).

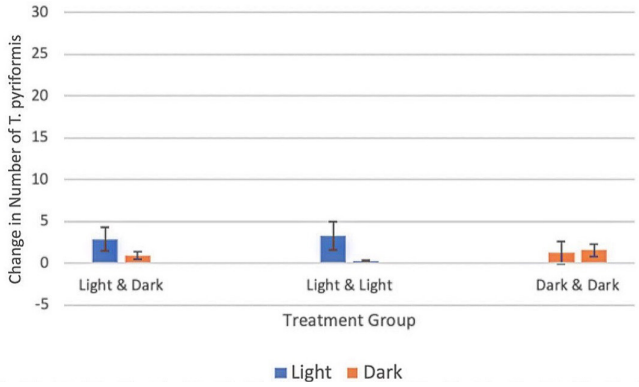

Figure 2: Exposure to light did not affect the distribution of *T. pyriformis*. Light exposure to two halves of a capillary tube was altered in the three treatments by a tin foil wrapping. The tubes exposed to light on both sides and those exposed to darkness on both sides were the control groups. Tubes were left under a light source for 30 minutes. The change in the average sum of three counts of organisms on each side of the capillary tube before and after exposure to its treatment was obtained and shown. Error bars indicating AAD are shown. All post-treatment and pre-treatment counts were not significantly different (*t*-test: $p>0.05$, n=3).

The relationship observed between light and the redistribution of *Euglena* confirms the previous research done (6). The phototactic response of *Euglena* is initiated by their PAC photoreceptors and red eyespot (6). In our experiment, we did not observe a phototactic response in the control groups of *Euglena*. The control groups were necessary to ensure that any distribution of the organisms was not the result of any external factors such as temperature or pressure but a direct result of the exposure to light or darkness. In the control groups the whole environment had optimal conditions for photosynthesis and respiration to occur, as in the case of the capillary tubes exposed entirely to light, or none of the environment had optimal conditions, as in the case of the capillary tube exposed entirely to darkness. However, it must be noted that *Euglena* are both heterotrophic and autotrophic (1). In this experiment, an external food source was provided in the respective living solutions of both organisms; therefore, the dark environment was still a suitable living environment for *Euglena*. However, the optimal condition for survival was in areas exposed to light, where light could be absorbed as a source of energy through photosynthesis in addition to the living solution. Alternatively, the absence of an effect on

T. pyriformis can be attributed to the fact that they are not autotrophic. Due to the heterotrophic nature of *T. pyriformis*, they cannot carry out photosynthesis and do not experience tangible benefits to exposure to sunlight because it is not a more optimal environment for their existence in relation to areas of darkness.

Future work could decrease the variability introduced by the experimental design. For instance, scoring the capillary tubes could decrease variation in tube separation. Additionally, decreased introduction of air bubbles could increase survivability of organisms in the capillary tubes, thus increasing the number of countable organisms per experiment. To decrease the air bubbles, for example, the tubes can be checked under the microscope before counts to establish whether it is necessary to refill it.

In regard to the broad implication of this study, it may be interesting to examine the response of photokinesis in *Euglena*. Further study could examine the principle of photokinesis by exposing *Euglena* to different intensities of light and then quantifying a potential change in the velocity of their movement. In terms of the larger ecosystem, this could provide insight into the use of photokinesis to determine the effects of different times of the year on vertical migration patterns.

Overall, this study shows that *Euglena* exhibit a phototactic response to light, which likely helps the organism find optimal environments for photosynthesis. Additionally, we found that the distribution of *T. pyriformis* remains unaffected by the presence of light, likely due to the heterotrophic nature of the organism that renders both darkness and light equally suitable for survival.

MATERIALS AND METHODS

A 50 µl capillary tube was filled with the mixture of *Euglena* and its growing solution or the mixture *T. pyriformis* and its growing solution by using a wire plunger that pulled in the organisms and their paired living solution, from either the beaker or test-tube, into the capillary tube through an adhesion-cohesion mechanism (**Figure 3**). The growing solution for *T. pyriformis* was catalog #132315 ordered from Carolina Biological. The growing solution for *Euglena* was a pea medium composed of water and split peas that provide the easiest long-term method to grow *Euglena*. After the capillary tube was filled, the ends were then sealed with a Blue Stik brand reusable adhesive putty**,** that trapped in the organisms and their solution to prevent spillage (**Figure 3**). During preliminary testing it was determined that adhesive putty was the best option to prevent leakage of the organisms and avoid the skewing of data. A Sharpie was then used to draw marks at the halfway point (4.5 mm), 1.5 mm, 3 mm, 4.5 mm, 6 mm, and 7.5mm of the capillary tube. Excluding the halfway mark, which was used as a controlled guideline to break the tube in half after exposure to its respective treatment, these measurements were used as guiding markings under the microscope to establish three different areas of microscopic view. After adjusting the microscope view so that the metric measurement was in the middle of the frame, a picture was taken using a smartphone attached to a microscope attachment (**Figure 4**). Photographs of the sections between 0 mm-1.5 mm, 3 mm-4.5 mm, and 6 mm to 7.5 mm were used to establish an initial count of organisms between all six of the markings. Three measurements were

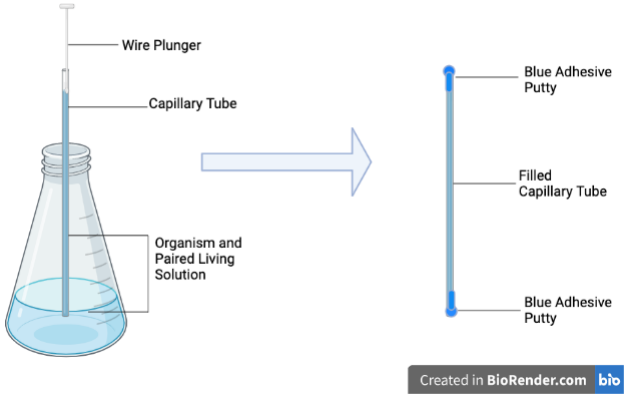

Figure 3: Apparatus used to collect organisms into capillary tubes. The process of filling each capillary tube with either *T. pyriformis* or *Euglena* consisted of three primary steps. First, we placed the capillary tube inside a beaker that was filled with the desired organism and its paired living solution. Next, we used a wire plunger to draw the mixed solution into the capillary tube. Finally, we sealed both ends of the capillary tube with a blue adhesive putty to prevent spillage.

taken between each of the previously described mark ranges on one half of the tube.

Tubes were then either covered until the halfway mark with tinfoil on one side and left uncovered on the other for the treatment exposing the tube to darkness and light, completely covered in tinfoil for the treatment exposing both sides of the tube to darkness, or left uncovered for the treatment exposing both sides of the tube to light (**Figure 5**). The process above was completed for three test tubes, one for each of the treatments, which were then placed under a light source that

Figure 4: Microscope and capillary tube set-up. Before and after exposure to light, dark, or light and dark treatments all capillary tubes were placed under a microscope which had an attachment that allowed for a smartphone to capture three microscopic images on both sides of the capillary tubes before and after exposure to their treatment group.

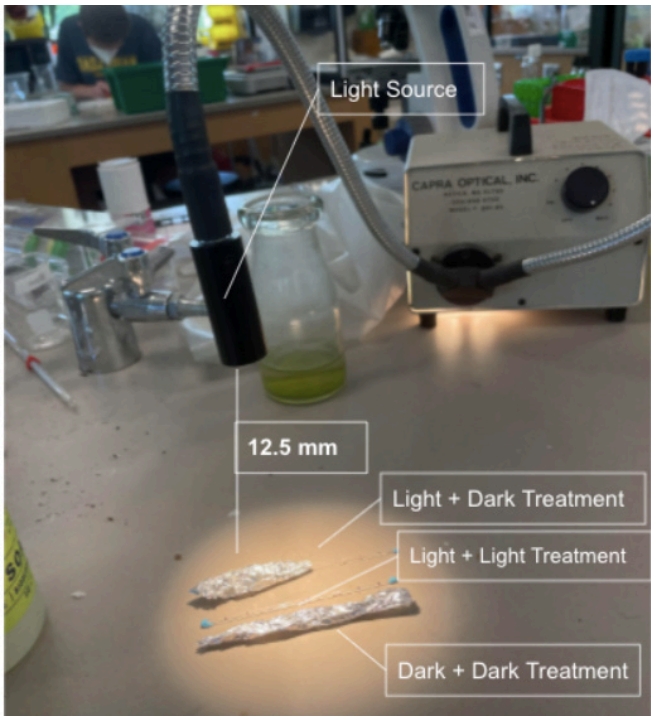

Figure 5: Light lamp and capillary tube set-up. Three capillary tubes, one exposed entirely to light, one exposed entirely to darkness because of its tin foil covering, and one exposed on one side to light and on the other to darkness, were placed under a single light source.

was positioned at a uniform distance of 12.5 mm above all the tubes, to control for the intensity of light throughout runs and trials (**Figure 5**). All tubes were left under the light source for 30 minutes. After the capillary tubes were removed from underneath the light lamp, each of the three tubes were broken by hand at the halfway point to prevent the redistribution of organisms after removal from their respective treatments. The tubes were sealed with a black Crayola brand molding clay and placed on a glass slide. After treatment with light, photographs of the sections between 0 mm-1.5 mm, 3 mm-4.5 mm were again taken to count the number of organisms in view. Photographs were taken six times for each of the three test tubes after the 30-minute exposure (**Figure 5**).

Three trials were conducted for each of the three runs (dark, light, and light and dark) by repeating the above process three times for the *T. pyriformis* and three times for the *Euglena.* As a control, the tubes were exposed on both sides to the dark treatment or on both sides to the light treatment

Data was collected, and the average sum of three counts on both sides of each test tube was calculated before and after exposure to light, darkness, or both. *T*-tests were performed on the average counts of the three runs prior to and after exposure to each trial for each half of the test tube to test the significance of changes in organism distribution.

Received: July 07, 2021
Accepted: November 24, 2021
Published: May 03, 2022

REFERENCES

1. "*Euglena*." *Encyclopedia Britannica*, Encyclopedia Britannica Inc., 5 May 2021, www.britannica.com/science/Euglena. Accessed 7 July 2021.
2. Cheng, Chao-Yin, *et al.* "Abundant and Diverse *Tetrahymena* Species Living in the Bladder Traps of Aquatic Carnivorous *Utricularia* Plants." Sci Rep, vol. 9, no. 13669, 2019, pp. 1-2, doi.org/10.1038/s41598-019-50123-1.
3. "Heterotrophs." *National Geographic*, National Geographic Society, 23 May 2019, www.nationalgeographic.org/encyclopedia/heterotrophs/. Accessed 14 Nov. 2021.
4. "Eyespot." *Encyclopedia Britannica*, Encyclopedia Britannica, 8 Jun. 2016, www.britannica.com/science/eyespot-biology. Accessed 7 July 2021.
5. Sigman, Daniel M., and Mathis P. Hain. "The Biological Productivity of the Ocean." Nature Education Knowledge, vol. 3, no. 10, 2012, pp. 1-9, www.marine.usf.edu/pjocean/packets/f01/f01u6p2.pdf.
6. Häder, Donat-P, and Mineo Iseki. "Photomovement in *Euglena*." *Advances in experimental medicine and biology*, vol. 979, 2017, pp. 207-235, doi:10.1007/978-3-319-54910-1_11.
7. "Phototaxis." *Merriam-Webster*. www.merriam-webster.com/dictionary/phototaxis. Accessed 7 Jul. 2021.
8. Häder, Donat-Peter, and Michael Lebert. *Photomovement.* E-book, Elsevier Science, 2001, pp. 302-305.
9. Dey, Ronit. "How Does Euglena Eat? (Nutrition in *Euglena*)." *Only Zoology*. onlyzoology.com/how-does-euglena-eat-nutrition-in-euglena/. Accessed 14 Nov. 2021.