Developing Teaching Materials using LED for a Phototaxis in *Euglena*

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SUMMARY

In order to demonstrate the occurrence of the phototaxis to high school students in a clear manner, an experiment using *Euglena* was improved. In this experiment, a compact apparatus was devised. Several Light-Emitting-Diodes (LEDs) containing white, blue, yellow, and red were used as the light source in the apparatus. The improved experiment using the newly devised apparatus was tested in four 10^{th} grade science classes. The students obtained good results. It was confirmed by students' reports that they could realize the phototactic phenomenon of *Euglena*. They also considered that there might be some relationships between the action spectrum for phototaxis and the absorption spectra of chlorophyll pigments.

Key Words: *Euglena*, LED, Phototaxis

I Introduction

Movement in response to light is called phototaxis. A well-known example of phototaxis can be demonstrated by a light trap. In order to kill pests, light traps have been used for many years. Students observe them in front of stores in the summer. In the current Japanese high school biology textbooks, phototaxis and related experiments are explained. In the current experiment, *Euglena* and a fluorescent lamp are used. *Euglena* has long been used as a teaching material[1] and is a popular type of plankton for use in the classroom[2]. Recently, a photo-avoidance substance was found in *Euglena gracilis*.[3]

In high school biology textbooks, in addition to the experimental materials mentioned above, color filters are used to examine the effect of light color on the phototactic movemenet of *Euglena*. Clear results can be obtained by light directly from a fluorescent lamp, but the results obtained by a fluorescent lamp passed through color filters are not clear. In order to obtain clear results of the light-quality experiment, we used a Light-Emitting Diode (LED) instead of the combination of fluorescent lamp and color filters. Currently, several LEDs emitting different color light (http://www.audio-q.com/index.htm) are available and are easy to obtain in Japan. Newly developed LEDs are cheap; they cost about two U.S. dollars each in Japan. To test the validity of the experiments, classes were conducted in Japanese high school science classes.

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II Materials and Methods

1. *Euglena*

Euglena gracilis strain Z (NIES-48 National Institute for Environmental Studies, Tsukuba, Japan NIES collection) was used in this study.

Euglena cells were heterotrophically grown at 25 degrees Celsius. One hundred ml of the medium used for the Euglena culture contained 100 mg of KH_2PO_4, 75 mg of $MgSO_4 \cdot H_2O$, 90 mg of NH_4Cl, 51 mg of sodium citrate, 500 mg of peptone, 500 mg of glucose, 1 mg of vitamin B_1, and 1 mg of vitamin B_{12} (pH 6.4). Under these conditions, the cells multiplied exponentially (doubling time being 12 h). Two weeks after inoculation, the cells were harvested under conditions of 13hours of light and 11 hours of dark. Cultures for *Euglena* may be obtained from any teaching materials company (for example, the Kyoto Kagaku Co., Ltd., in Japan) for approximately 20 U.S. dollars.

2. LED

Four LEDs were used for this study. They were white (wavelength 400-750nm), blue (470nm), yellow (590nm) and red (660nm). These LEDs cost approximately two U.S. dollars each and were purchased through the Internet (http://www.audio-q.com/index.htm). The circuit for these LEDs were obtained from the same Internet site for approximately 10 U.S. dollars; however, the circuit could be fabricated by using resistors and a plate. In such a case, the cost would be approximately two U.S. dollars.

3. Device for the phototaxis experiment

Figure 1 shows a cross section of the device for the phototaxis experiment; the device irradiates exclusively LED light. First, a Petri dish with a 5 cm diameter containing 3 ml of the *Euglena* culture (*Euglena* number 6×10^6 cells) was placed on a desk. Next, the Petri dish was covered with a black paper box(①) that had a hole in the center. Finally, the black paper box was covered with another black paper box(②) with an LED attached. Four devices (white, blue, yellow, and red) were made. The intensities of the LEDs were regulated to be 250 lx. The light intensity was measured by a dosimeter (Topcon IM-2D).

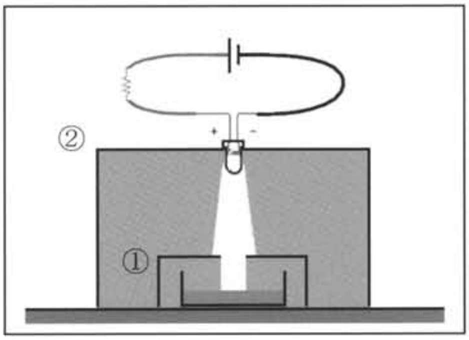

Figure 1. Cross section of the device used for the phototaxis experiment.

4. Phototaxis experiment procedure

After gently stirring the Petri dish, the LED was irradiated on *Euglena* for 15 minutes by using the device. If the class time is sufficient, we recommend 30 minutes of irradiation. However, good results can be obtained with an irradiation time of 15 minutes.

5. Class

(1) Object: Four 10th grade classes. There were 44 students in each class.

(2) Activity:

1st period

Learning about the concept of phototaxis.

Observation of *Euglena* with a light microscope.

Phototaxis experiment of *Euglena* using white light.

2nd period

Learning about electromagnetic wave.

Learning about absorption spectra of chlorophyll a and b.

Phototaxis experiment of *Euglena* by blue, yellow, and red light.

Learning about paraflagellar body as the photoreceptor of *Euglena*.

Phototaxis experiments were conducted by 11 groups of 4 students each.

Ⅲ Results and Discussion

1. The laboratory experiment

Figure 2 shows the results of the preliminary laboratory experiment. The column shows the irradiation time of the various lights on *Euglena*. The row shows the results with various LED lights. A green cluster can be observed in the center of the Petri dish under white and blue light in 20 and 30 minutes, respectively. These findings indicate that *Euglena* cells move toward light. Biological responses regulated by blue light are widespread among the plant and fungal kingdom.[3]

Therefore, *Euglena* might move toward a blue LED light. Since white LED lights contain blue, *Euglena* might also move toward white light.

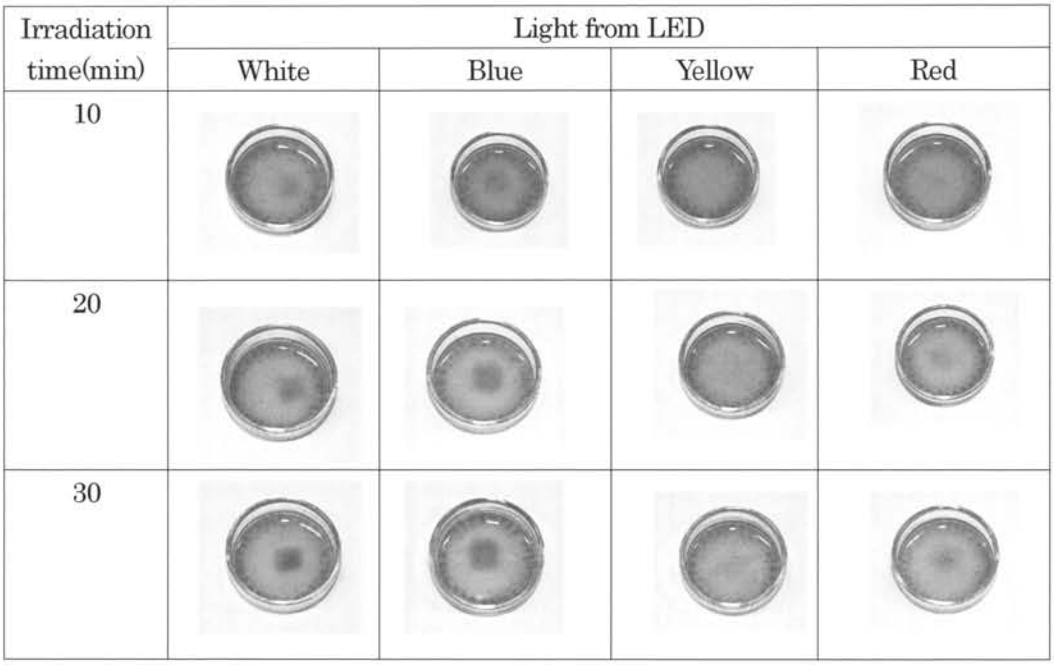

Figure 2. Results of the preliminary laboratory experiment.

A photoreceptive organ of *Euglena* is a paraflagellar body that contains flavin.[4] The absorption spectra of flavin have an absorption band on 380 nm and 450 nm. The results of the preliminary laboratory experiment are consistent with this information.

However, as students do not study flavin, they lack this knowledge and cannot predict the experimental results in this way. A feasible prediction for them is the prediction using the absorption spectra of chlorophyll a and b explained in their biology textbooks. The absorption spectra of chlorophyll a and b have an absorption band from 420 nm to 460 nm[5]. The absorption band of flavin is similar to that of chlorophyll. The absorption band of chlorophyll is available to the students.

It can also be observed that there is a green cluster in the center of the Petri dish that was illuminated by a red LED light in 30 minutes. This result shows that *Euglena* moves toward the red light. However, this result was not stable, and a cluster could sometimes be observed in the center of the Petri dish. The cluster could not always be observed in the center of Petri dish.

2. Class

1^{st} period

While studying phototaxis, some students noticed examples of phototaxis in their daily life. A light trap is one example. In this lesson, the teacher explained some other taxtic movements of organisms, such as chemotaxis, geotaxis, rheotaxis and electrotaxis.

While observing *Euglena* under a light microscope (magnification 150, 600), the students observed the ellipsoidal shape and the rapid movement of *Euglena*. They also observed the green chloroplasts in *Euglena*. Though they tried to observe the flagellum of *Euglena*, they did not succeed. Usually, it is difficult to observe a flagellum under a light microscope.

As the Japanese science curriculum includes the observation of plankton in a pond for lower secondary schools, the students may have observed *Euglena*. However, they seemed to enjoy the observations. The students said that the shape of the *Euglena* was attractive and the changing shape was peculiar.

Table 1 shows the results of the student experiments on phototaxis carried out with white LED light. Phototaxis was confirmed by the 10 of the 11 students groups in the first class. The cause of the one failure was attributed to the fact that the Petri dish had been moved before the results were examined. In the other 3 classes, phototaxis was confirmed by all groups.

Figure 3 shows students observing the results of phototaxis of *Euglena* under a white LED.

Table 1. The experimental results of student laboratories in 1st period (white LED). The numbers indicate the groups.

Class	1st	2nd	3rd	4th
Success	10	11	11	11
Failure	1	0	0	0

Figure 3. Students observing phototaxis of *Euglena* under a white light.

The students' impressions were as follows.

"As *Euglena* took a square shape, I was surprised. I think that the phototactic property of *Euglena* is strong."

"Our group's result was not a square shape. It is possible that someone might have shaken the Petri dish."

"Before this experiment, I thought that moving toward the light was a property unique to insects. I have now realized that other animals also have phototactic properties."

"I could understand the phototactic property in *Euglena*. I wonder what could happen if the intensity of light were stronger."

"I guess *Euglena* has a photosynthetic function and a phototactic property."

The students showed a positive attitude toward the experiment. They confirmed the phototactic property of *Euglena*. They thought about the cause of the failure of their experiment. Moreover, they had a question for phototaxis.

Euglena has positive phototaxis under weak light, and *Euglena* has a negative phototaxis under strong light. The threshold value coincides with the intensity of light in a maximum photosynthetic rate.

The results obtained in this study definitely indicate that phototaxis experiments with *Euglena* and white LED help students understand the concept of phototaxis.

2nd period

Table 2 shows the students predictions before the phototaxis experiment in the 2nd period. Nearly one half of the students predicted that *Euglena* shows phototaxis to blue and red light. Their prediction might come from the absorption spectra of chlorophyll a and b.

About 20 percent of the students predicted that *Euglena* moves toward the yellow light. Such predictions were based on their idea that yellow is a light color, and *Euglena* is most responsive to that type of light.

The student predictions are noted in the following:

"*Euglena* has chlorophyll that absorbs blue and red and reflects yellow light. Therefore, I predict that *Euglena* will move toward the blue and red light."

"As yellow light resembles sunlight, I predict that *Euglena* moves toward the yellow light."

Table 2. Students' predictions.

Prediction	Number	%
Blue and Red	71	46
Yellow only	16	10
Blue and Yellow	15	10
Blue, Yellow, and Red	12	8
Yellow and Red	10	6
Others	30	19
Sum	154	100

As shown in Table 3, students of all groups could confirm phototaxis under blue LED light. The blue LED light led to very clear results in all groups. As stated previously, under a red LED light, the results were not stable: some groups could confirm phototaxis and some groups could not. The students could obtain similar results to those of our experiments in the laboratory. The experimental results of the 1st period and the 2nd period indicate that it is easy to arrange for the students to conduct these experiments.

Table 3. Experimental results in the 2nd period (the difference among Blue, Yellow and Red LED). The numbers show the number of groups.

Class	1st	2nd	3rd	4th
Blue and Red	6	5	2	4
Blue only	5	6	9	7
Yellow	0	0	0	0
Failure	0	0	0	0

The students' discussions were as follows.

"I understand that *Euglena* chooses the blue light from among the blue, yellow, and red light. I guess that *Euglena* needs the blue light for its photosynthesis."

"I want to know the response of *Euglena* to various light colors. Although our group failed in the previous experiment and I regretted it, I am happy that we succeeded in this experiment."

"I am glad to know the different responses of *Euglena* to different shades of light. I want to study the response to light of other living things."

"In comparison with previous experiment, it was difficult for me to predict the results. However, as good results were obtained, I enjoyed the experiment."

"Though our results did not coincide with my prediction, I am happy to have learned form the experiment."

"In spite of being a microorganism, *Euglena* reacts to the differences in the shades of light. I admire *Euglena*."

"Since the living things contain chlorophyll need light for photosynthesis, I was surprised to learn that they respond to wavelengths."

The students seemed to have confidence in their experiments. The experiment was very motivational. They recognized that the microorganisms are active and had survival mechanisms.

The results indicated that the students learned about phototaxis, photosynthesis, and light and improved their problem-solving skills. It was easy for high school students to follow the procedures reported here.

However, it was a little difficult for the students to predict the results in 2nd period. Some students did not understand the meaning of absorption spectra. In order to understand the concept of absorption spectra, the students might need to study physics. Wavelengths were unfamiliar to some students. The experiment could be improved by combining aspects of physics and biology.

Finally, we conclude that this experiment would be useful for high school students.

Ⅳ Conclusion

This paper is a report of a newly devised experiment on phototaxis and an examination of its impact on learning. The devised experiment provides clear experiment results that may be obtained in a classroom setting as easily as in a laboratory setting.

Moreover, the experiment could attract students and facilitate active learning. We hope this promotes teaching and learning in biology classrooms.

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