

ENV101 – LABORATORY: **EFFECTS OF ULTRAVIOLET LIGHT EXPOSURE ON** **FRESHWATER PROTIST SURVIVAL**

ABSTRACT

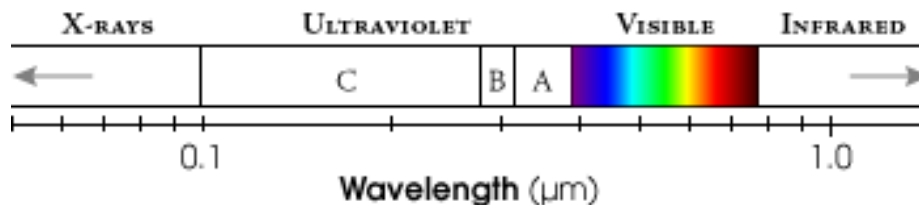
The Sun provides heat and light (energy), which are essential for life on Earth. The radiated energy is in a wide range of wavelengths, most of which are invisible to our eyes. The shorter wavelengths contain more energetic radiation and have the potential to harm living organisms. The ultraviolet (UV) wavelengths fall just below the visible wavelengths that we see; they are also the wavelengths that can cause cellular damage to living organisms. The shortest of these rays in sunlight, known as UV-B (280 nm to 320 nm) can cause damage to DNA. In this lab, you will perform an experiment that will demonstrate the lethal effects of UV light on cell function using freshwater Protista (plankton).

TERMS AND CONCEPTS

- Plankton
- Photosynthesis
- Energy
- Nucleus
- DNA damage
- Enzyme
- Ultraviolet light

BACKGROUND/INTRODUCTION

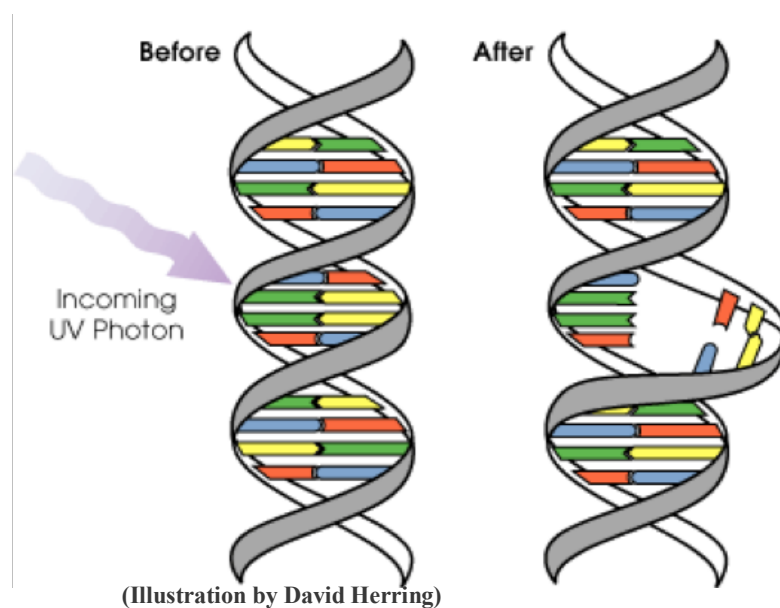
The star around which our planet orbits radiates energy in a wide range of wavelengths, most of which are invisible to human eyes. The shorter the wavelength, the more energetic the radiation, and the greater the potential for harm. *Ultraviolet (UV)* radiation that reaches the Earth's surface is in wavelengths between 290 and 400 nm (nanometers, or billionths of a meter). This is shorter than wavelengths of visible light, which are 400 to 700 nm.



UV radiation from the sun has always played important roles in our environment, and affects nearly all living organisms. Biological actions of many kinds have evolved to deal with it. Yet UV radiation at different wavelengths differs in its effects, and we have to live with the harmful

effects as well as the helpful ones. The longer UV wavelengths (320-400 nm, called **UV-A**) plays a helpful and essential role in formation of Vitamin D by our skin, and yet plays a harmful role in that it causes sunburn on human skin and cataracts in our eyes. The incoming radiation at shorter wavelengths, 290-320 nm, falls within the **UV-B** part of the electromagnetic spectrum. UV-B causes damage at the molecular level to the fundamental building block of life—**deoxyribonucleic acid (DNA)**.

DNA readily absorbs UV-B radiation, which commonly changes the shape of the molecule in one of several ways. The illustration below shows one such change in shape resulting from exposure to UV-B radiation. Changes in the DNA molecule often mean that protein-building enzymes cannot “read” the DNA code at that point on the molecule. As a result, distorted proteins can be made with reduced function, and/or cells can die.



But living cells over millions of years of evolution in the presence of UV-B radiation have developed a variety of repair mechanisms to ‘fix’ their DNA. Special enzyme arrives at the damage site, removes the damaged section of DNA, and replaces it with the correct and functional nucleotides (based on information elsewhere on the DNA molecule). This makes

DNA somewhat resilient to damage by UV-B. The amount of this resiliency varies among organisms.

Living things on earth are also protected in part from excessive amounts of UV radiation by a chemical called **Ozone**. A layer of ozone in the upper atmosphere absorbs most UV radiation and prevents most of it from reaching the Earth. Without the layer of ozone in the stratosphere to protect us from excessive amounts of UV-B radiation, life as we know it would not exist. Yet

since the mid-1970s, human activities have been changing the chemistry of the atmosphere in a way that reduces the amount of ozone in the stratosphere (the layer of atmosphere ranging from about 11 to 50 km in altitude). This means that more ultraviolet radiation can pass through the atmosphere to the Earth's surface, especially near the poles and nearby regions during certain seasons of the year. Scientific concern over ozone depletion in the upper atmosphere has prompted extensive efforts to assess the potential damage to life on Earth due to increased levels of UV-B radiation. Some effects have been studied, but much remains to be learned.

Watch Video: Climate 101:Ozone Depletion : <https://youtu.be/aU6pxSNDPhs>

With regard to plants, UV-B impairs photosynthesis in many species. Overexposure to UV-B reduces size, productivity, and quality in many of the crop plant species that have been studied (among them, many varieties of rice, soybeans, winter wheat, cotton, and corn). Similarly, overexposure to UV-B impairs the productivity of *phytoplankton* in aquatic ecosystems. Scientists have found it affects enzyme reactions that conduct fundamental biological functions such as cellular division and molecular repair mechanisms. Since some species are more vulnerable to UV-B than others, an increase in UV-B exposure has the potential to cause a shift in species composition and diversity in various ecosystems. Because UV-B affects organisms that move *nutrients* and *energy* through the biosphere, changes in their activities may alter biogeochemical/**trophic** cycles in various ecosystems. For example, reducing populations of phytoplankton would significantly impact the world's carbon cycle, since phytoplankton store huge amounts of carbon, and impact trophic feeding systems since phytoplankton are the base primary producer for all marine ecosystems.

LABORATORY EXERCISE

Pure water is transparent to UV, so in clear waters ecosystems (e.g. mid-ocean, alpine lakes) much of the upper layer is exposed to UV. One of the main biological impacts of UV exposure on aquatic ecosystems is a reduction in the rate of photosynthesis. This has a direct effect on primary productivity and, since different species may vary in their sensitivity to UV exposure, may eventually affect biodiversity. Heterotrophic microorganisms (zooplankton such as ciliates, planktonic larvae) are also sensitive to UV exposure. Genetic material (DNA) and cellular components (proteins, membranes) are damaged, causing damage or mortality when exposure is

sufficient.

In this lab we will be testing the effects of UV light exposure on survivorship of plankton by exposing slides with drops of water containing planktonic species to differing amounts of UV light. We will be observing the activity levels before exposure and then after increasing amounts of exposure.

Please watch the linked Youtube video to see what your slide(s) may have looked like. The smaller rapidly swimming protists are the euglena and the larger peanut shaped swimmers (moving a bit more slowly) are the paramecium. (See image on last page of lab)

“Protists – Amoeba, Euglena, Paramecium : <https://youtu.be/bNTQLqWEpXU>

The pond scum was a mix of single and multicellular microscopic organisms, some of which can be seen in this Youtube video.

How Microscopic Hunters Get Their Lunch : <https://youtu.be/io731XY8fH8>

Now:

First, identify the primary question we are addressing.

PRIMARY QUESTION: _____

Write out a clear hypothesis on how you think yeast’s ability to respire will be affected by temperature based on the reading above and your knowledge.

TESTABLE HYPOTHESIS: _____

Read over the procedures below so you are familiar with the overall experimental conditions and make predictions as to your primary results.

PREDICTED RESULTS: _____

MATERIALS

- Euglena/paramecium/eutrophic pond water
- ~~Microscope slides with grids~~
- ~~UV lights~~
- ~~Stopwatch~~
- Data from previous classes

PROCEDURES

1. ~~Set up microscope and use prepared slides to practice microscope use and handling using only the lowest and medium power objective.~~
2. ~~Prepare a wet slide of the *Euglena* by placing one (~~ONE (1)~~) drop in the center of the grid on the slide.~~
3. ~~Gently place a cover slip over the drop as demonstrated by your instructor.~~
4. ~~Place slide and microscope and using the scanning (Red ~~lowest power~~) objective to observe plankton activity. Find an active zone on the slide on the grid, where you can see organisms passing across a single grid/quadrat of your choice.~~
5. ~~Set your stopwatch to 15 seconds.~~
6. ~~Start the stopwatch and count how many organisms move within or pass through your grid/quadrat of choice during the 15 seconds. At the end of trial write down the number in the data table below in the area marked "Grid 1", in the "No UV Light" column.~~
7. ~~Move the slide to another "Grid", and repeat the process of counting moving *Euglena* in the new grid. Enter your data in the "Grid 2" under "No UV Light".~~
8. ~~Repeat this process three (3) more time, until the column is full.~~
9. ~~Also write down any general observations you have about the slide (below the table)—levels of activity, layers, and types of organisms you see.~~
10. ~~Carefully remove and clean your slide to begin a new preparation with a new drop from the sample jar.~~
11. ~~Prepare a wet slide of the *Euglena* by placing one (~~ONE (1)~~) drop in the center of the grid on the slide.~~
12. ~~Carefully bring the slide over to the UV lights and **Following the instructor's directions for UV exposure of the slides.**~~
13. ~~Place the slide under the turned off UV light, and make sure your hands are clear of the lights.~~
14. ~~Turn the light on for 15 seconds, then it turn off.~~
15. ~~After the light is off for several seconds, then carefully remove the slide, place a cover slip over the drop, and return it to the microscope.~~
16. ~~Then repeat steps 5 and 10.~~
17. ~~Then bring the slide back to the UV lights and this time expose it for another 30 seconds.~~
18. ~~Then repeats steps 11 through 16, with increasing amounts of UV exposure, as stated in the tables.~~
19. ~~Repeat these procedures until the table is full.~~
20. ~~When you are done, rinse and dry your microscope slide.~~
21. ~~Calculate the means (average) for each treatment.~~
22. ~~Now repeat steps 2 to 21 using the *Paramecium* live specimens and then again using the "**Pond Water**" live specimens (as time permits).~~
23. ~~When done clean and return the slides to the front table and the microscopes to their cabinets.~~
24. ~~Finally, use the calculated means for each treatment to graph your results.~~

PROCEDURES 2

1. You will be provided with data from previous semesters and a brief description of the samples.
2. You will calculate a mean (average) for each of the conditions (No UV, 15 seconds UV, 30 seconds UV, 45 seconds UV, 60 seconds UV) for each group of organisms.
3. You will create a graph (or three) comparing the mean exposure levels for each of the types of organisms.
4. Then as homework due, you will 1) provide your Question/Hypothesis/Predictions from above, 2) answer the questions below (p. 9)
5. This will all be due within 24 hours of the end time for your lab section.

DATA COLLECTION TABLE 1. - EUGLENA SAMPLES (IF AVAILABLE)

Eugleina	Sample	No Uvlight	15 Sec UV	30 Sec UV	45 Sec UV	60 Sec UV
Eugleina	1	20.8	16	9.8	6.6	1
Eugleina	2	18.8	18.2	6.2	1	0
Eugleina	3	24.4	20.2	9.8	4	2.4
Eugleina	4	22	24.2	15.4	10.8	0.8
Eugleina	5	35.8	26.8	28.2	15	6.6
Eugleina	6	28	32.2	17.8	2.2	0.2
Eugleina	7	26.2	28.6	24.4	3.9	0.2
Eugleina	8	88.2	21.2	11.8	13.2	47
Eugleina	9	23.6	19.4	5.8	0	0
Eugleina	10	23.4	14.2	0.2	0.4	0
Eugleina	11	13.4	3.2	1	0.2	0
Eugleina	12	2.2	2.6	1.8	0	0
Eugleina	13	20.8	16	9.8	6.6	1
Eugleina	14	18.8	18.2	6.2	1	0
Eugleina	15	24.4	20.2	9.8	4	2.4
Eugleina	16	22	24.2	15.4	10.8	0.8
Eugleina	17	35.8	26.8	28.2	15	6.6
Eugleina	18	28	32.2	17.8	2.2	0.2
Eugleina	19	26.2	28.6	24.4	3.9	0.2
Eugleina	20	88.2	21.2	11.8	13.2	47
Eugleina	21	23.8	20.6	6	1.8	1.6
Eugleina	22	40.2	46.2	11	0	0
Eugleina	23	44.4	39.2	22.6	10	2.4
Eugleina	24	25.2	20.8	14	8.6	1.8
Eugleina	25	4.4	25.6	27.6	1	0.4
Eugleina	26	24.8	24	11	0.6	5
Eugleina	27	5.2	3.2	1.4	0	0
Eugleina	28	9.8	13	7.8	0.8	0.6
Eugleina	29	10.2	10.4	6	1.4	0.2
Eugleina	30	18.2	5	4.8	2.6	1.8
Eugleina	31	18.2	8.6	4.2	0	0.2
Eugleina	32	10.4	8.2	5	0.2	0
Eugleina	33	15.6	17	19.2	0.2	0
Eugleina	34	37.6	39.8	13.4	6.2	0.2
Eugleina	35	21.4	30.4	0	0	0
Eugleina	36	32.2	23.4	1.2	2	0.2
Eugleina	37	32.2	19.6	0	0	0
Eugleina	38	44.4	39.2	22.6	10	2.4
Eugleina	39	25.2	20.8	14	8.6	1.8
Eugleina	40	4.4	25.6	27.6	1	0.4
Eugleina	41	24.8	24	11	0.6	5
Eugleina	42	24.7	27.4	18.8	5.05	2.4
Means						

- THESE SAMPLES WERE DRAWN FROM THE TEST TUBES WITH HIGHEST GROWTH RATES FROM THE PREVIOUS LAB (EUTROPHICATION), EACH NUMBER IS THE AVERAGE OF FIVE COUNTS. EUGLENA ARE CONSIDERED ‘PHYTOPLANKTON’ BECAUSE THEY USE PHOTOSYNTHESIS TO OBTAIN ENERGY.

DATA COLLECTION TABLE 2. - PARAMECIUM SAMPLES (IF AVAILABLE)

Paramecium	Sample	No Uvlight	15 Sec UV	30 Sec UV	45 Sec UV	60 Sec UV
Paramecium	1	6	1.2	0.6	0	0.4
Paramecium	2	2.8	1	0.8	0.8	0
Paramecium	3	8	2.6	0.4	2.4	0
Paramecium	4	1.4	1.8	2.8	0.2	0
Paramecium	5	3.4	1.6	0.4	0.2	0
Paramecium	6	7.4	5	2.2	9	2.2
Paramecium	7	8	4	2.6	1.8	0.6
Paramecium	8	5.2	2.2	1	0	0
Paramecium	9	2.8	2.4	4	3.2	0.8
Paramecium	10	2	2.8	2.4	2	0.8
Paramecium	11	6.8	1.8	3.6	3.6	2.8
Paramecium	12	2	4	0.6	0.8	0.4
Paramecium	13	9	0.2	6.4	4.4	0
Paramecium	14	8.2	7.4	4	4	4
Paramecium	15	6.2	0.4	0	0	0
Paramecium	16	5.2	1.4	0	0	0
Paramecium	17	12.6	8.4	1	0	0
Paramecium	18	13.2	8.8	9.2	1.4	1.4
Paramecium	19	1	0.8	1.4	1.2	0
Paramecium	20	1.2	13	0.6	0	0
Paramecium	21	1.4	1.6	0.4	0	0.4
Paramecium	22	4.4	11	1	0.4	0.4
Paramecium	23	3.4	1.2	0.4	0	0.2
Paramecium	24	5	2	1.8	1	0.6
Paramecium	25	1.6	28.2	2.6	4.4	3.4
Paramecium	26	1.2	1.2	0.6	0.8	0.4
Paramecium	27	1.8	1.6	2.8	0	1.4
Paramecium	28	0.4	2	0.2	0	0
Paramecium	29	0.6	1.4	0.8	0.8	12
Paramecium	30	7.4	17	1.2	0	0.2
Paramecium	31	1.6	0.2	0.8	0.2	0
Paramecium	32	3.2	2.6	1.6	0.8	0.2
Paramecium	33	3.4	2	2.2	0.4	0
Paramecium	34	7.2	4.8	3.4	1	0.8
Paramecium	35	5.2	3.8	2.8	2.4	1
Paramecium	36	0.6	3.4	0.4	0	0
Paramecium	37	8.6	3.4	3	1.5	0.6
Paramecium	38	3.4	2	2.2	0.4	0
Paramecium	39	1.2	7.2	3.2	2	3
Paramecium	40	2.6	2.8	1.2	0	0
Paramecium	41	14.4	4.6	2.6	4.8	1
Paramecium	42	9	14	3.8	16.6	5.8
Means						

- THESE SAMPLE COUNTS ARE FROM THE SAME COUNTING PROCEDURE AS THE EUGLENA, HOWEVER THESE ARE LARGER ORGANISMS LIVING AT LOWER DENSITIES. THEY ARE CONSIDERED ‘ZOOPLANKTON’ BECAUSE THE MOSTLY EAT OTHER PLANKTONIC ORGANISMS.

DATA COLLECTION TABLE 3. - MIXED POND WATER SAMPLE

Pond Muck	Sample	No Uvlight	15 Sec UV	30 Sec UV	45 Sec UV	60 Sec UV
Pond Muck	1	1.2	1	1.8	2.6	0
Pond Muck	2	3.4	11.2	0.8	1.6	0.2
Pond Muck	3	6.4	2.2	2.2	1.4	0.6
Pond Muck	4	2.6	3.4	0.6	0.8	0.4
Pond Muck	5	0.4	0.8	0.6	0	0
Pond Muck	6	3.2	1	1.4	1.4	0
Pond Muck	7	2	2.2	0	0	0
Pond Muck	8	1.4	2.6	1.8	0.4	1.2
Pond Muck	9	2.8	2.2	1	1	0.4
Pond Muck	10	1.8	1	1.6	1.4	0.4
Pond Muck	11	6.2	0.4	1.2	0	0
Pond Muck	12	6.8	2	2.2	1	0
Pond Muck	13	0	0.2	0.4	0.2	0
Pond Muck	14	3.4	1.8	5.4	0.8	1.8
Pond Muck	15	4.4	3.8	1.6	0.6	0
Pond Muck	16	6.8	10	9.4	5.4	7.8
Pond Muck	17	1.4	0.8	2.2	0.4	0
Pond Muck	18	9.6	4.4	3.6	2.4	1.8
Pond Muck	19	11.4	4.4	3.6	1.6	5.8
Pond Muck	20	0.4	0	0.4	0.6	0
Pond Muck	21	0.8	0.6	0.2	0	0.6
Pond Muck	22	5.6	2.6	0.2	0	0
Pond Muck	23	0.4	0.4	0.2	0	0.2
Pond Muck	24	7.8	5.2	1	0.2	0.2
Pond Muck	25	0.4	0.2	0.4	0	0
Pond Muck	26	1.2	1.4	2.2	1.2	1
Pond Muck	27	6.8	2	2.2	1	0
Pond Muck	28	5.2	4.4	1.8	2	2
Pond Muck	29	4.6	4.6	2.6	1.8	1.6
Pond Muck	30	1.6	1.2	3.4	1	2.8
Pond Muck	31	4.2	2	1.4	2	2
Pond Muck	32	2.8	2.2	1	1	0.4
Pond Muck	33	5.4	2	5.4	1	2
Pond Muck	34	1	1.4	2.6	1.8	1.6
Pond Muck	35	2.4	3	4.4	1	0
Pond Muck	36	2.6	3.4	0.6	0.8	0.4
Pond Muck	37	1.8	1.6	0.8	0.4	0.8
Pond Muck	38	1	2.4	1.6	1	0.2
Pond Muck	39	1.8	8	5	6.8	5.2
Pond Muck	40	2.2	2.2	4.6	2.6	2
Pond Muck	41	1.8	1.6	1.2	1.6	2
Pond Muck	42	1.84	1.4	2.8	4	2.6
Means						

- THE POND MUCK WAS FROM THE BOTTOM OF THE SMALL FISH TANK IN OUR LAB. STUDENTS WERE ONLY COUNTING THE LARGER, MULTICELLULAR ORGANISMS (ROTIFERS, WORM-LIKE CRITTERS), THE RELATIVELY BIG STUFF. BUT THEY WERE ABLE TO OBSERVE MANY OF THE SAME ORGANISMS THAT WERE IN THE PREVIOUS SAMPLES. SAME SAMPLING PROCEDURE, BUT LOWER NUMBERS TO START WITH. WE NEVER KNOW WHAT WILL BE FOUND.

QUESTIONS FOR ANALYSIS

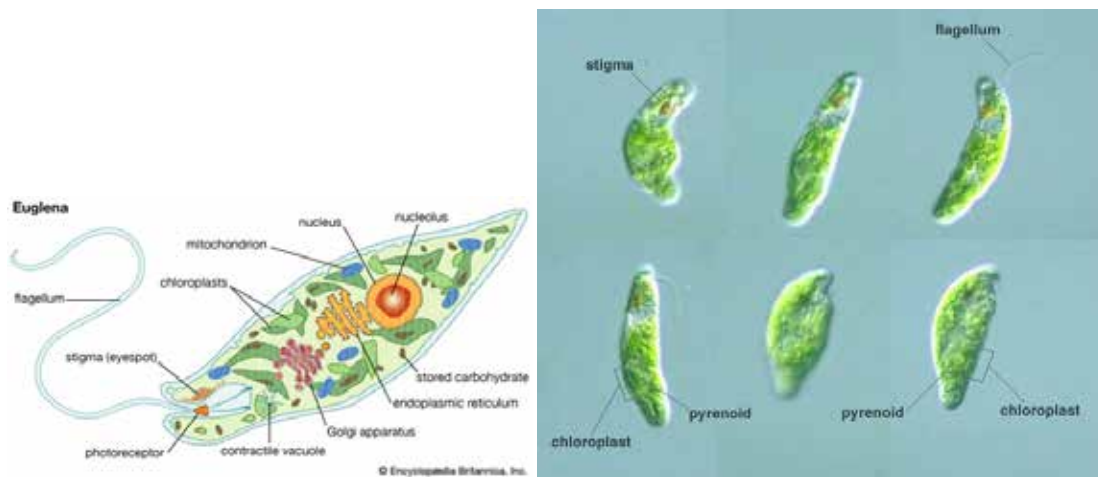
Graph your results (the means) using column graph. (Treatment is the Independent variable, and means is the dependant variable.) You may use your textbook and other references if needed.

After graphing your results, answer the following questions.

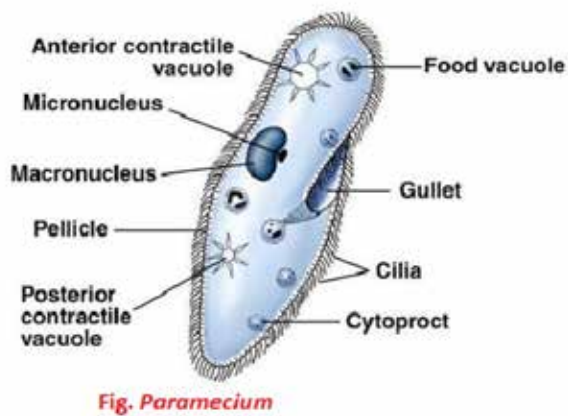
1. Our basic assumption was that increased UV exposure will reduce the viability of the freshwater plankton, and thus their level of activity. Were these your results? Explain for each type of organisms how your hypothesis supported, or not.
2. Was there a difference between the green algae (euglena and other) and the zooplankton (paramecium or other micro-organisms) in how they reacted to the UV light? Explain why you think this happened? (Or why it did not happen, if they responded the same.)
3. What about the mixed pond water planktonic organisms, do you think everything in the sample reacted the same way? Explain why they should (or should not) all react to the UV light in the same manner.
4. What other experimental factors/procedures may have affected your results?
5. Plankton are the basis of most aquatic food chains. Carefully explain why increasing levels of UV light passing through the atmosphere (in areas with a reduced “ozone layer” - like the Antarctic) impact the *trophic* levels in an aquatic ecosystem?
6. Would the impacts of increased UV exposure be different for Marine (ocean) systems than for freshwater ecosystems? Why?
7. What could be the impacts for terrestrial organisms?
8. What about plant cells? What adaptations might plants have to reduce the impact of UV light damage?
9. Many Marine invasive species travel in the ballast water of cargo ships. How could we use our newly gained knowledge about UV light to reduce the transport of invasive planktons?
10. Explain how the loss of oceanic phytoplankton would affect atmospheric carbon levels, and the carbon cycle.

HOMEWORK: 1) provide your Question/Hypothesis/Predictions from p. 4, and answer all 10 + questions above. All Answers should be typed with complete sentences and carefully thought out responses. They are due, along with three excel graphs within 24 hours of your lab period.

Euglena



Paramecium



Rotifers : https://youtu.be/FRZ64_Izf_8