INQUIRY & INVESTIGATION

Population Growth in *Euglena*: A Student-Designed Investigation Combining Ecology, Cell Biology, & Quantitative Analysis

CHRISTINE OSWALD,
STEPHEN KWIATKOWSKI

Abstract

We describe the use of Euglena gracilis *for student-designed investigations of environmental factors that affect population growth of this unicellular flagellate. Each group of students can choose a topic of interest to them, but within the confines of a simple cell culture system that requires little space for each student group, and at a fairly low cost. Students are provided with stock cultures of* E. gracilis*, along with detailed instructions on preparing control medium and counting cells using a hemocytometer. Students then design their own experiments to test factors that possibly affect population growth over several weeks.*

Key Words: *Cells; population growth;* Euglena*; cell culture; scientific method.*

The *National Science Education Standards* (NSES) stress "science as inquiry" as crucial to science education at all levels (National Research Council, 1996). Often, a tension exists between allowing students the freedom to pursue questions entirely of their own design and the practical considerations associated with materials, space, and the instructor's ability to keep track of many diverse experiments, particularly in a class with multiple sections. Here, we describe the use of *Euglena* for student-designed investigations of environmental factors that affect population growth. This project allows each group of students to choose topics of interest to them, but within the confines of a relatively simple cell culture system that requires little space for each student group, and at a fairly low cost. We have used this as a term-long project in an introductory college course for biology majors (six sections of 24 students), but it could be adapted for use in high school classes as well.

The NSES specifies several fundamental abilities and concepts that underlie the "science as inquiry" standard.

The NSES specifies several fundamental abilities and concepts that underlie the "science as inquiry" standard. This project addresses several of them, including identification of questions that guide scientific investigation, design and performance of scientific investigations, communication and defense of a scientific argument, and use of mathematics to improve investigations. The NSES content standards state that "mathematics is essential in scientific inquiry." The repeated use of calculations throughout data collection and the application of population-ecology mathematical models to the final data set address this standard in a direct way. Students collect a fairly large amount of data for statistical analysis at whatever level the instructor thinks appropriate for the students' grade level. The NSES content standards also refer to the relationship between the techniques and technology used and the quality of the results obtained. In this project, students have ample opportunity to assess their data-collection techniques, particularly sampling procedures, and discuss how they may influence the outcome.

An additional educational benefit of this project stems from its long-term nature. Students collect data from multiple replicates over several weeks. This provides them with a more realistic taste of scientific research than 3-hour lab exercises do. This also generates a great deal of raw data that must be organized, tabulated, and analyzed using a spreadsheet.

Students develop questions ranging from straightforward investigation of one particular nutrient on *Euglena* growth to more complex questions about the interactions of multiple factors, such as light and carbon source. The effect of pollutants known to be harmful in aquatic ecosystems is a popular choice. This is also a very appropriate choice, given the importance of *Euglena* as an organism for bioassay of environmental toxins (Danilov & Ekelund, 2001; Streb et al., 2002; Ahmed & Häder, 2010). This practical application gives real-world meaning to their experiments and enhances the interest of many students in the project.

In addition to the experience of designing, conducting, and reporting on their own scientific investigation, students gain specific knowledge and skills in the following areas:

- Techniques for handling cultures
- Use of micropipettes
- Preparation of dilutions and performance of calculations related to dilutions
- Counting cells in a sample and calculation of an estimate of the population density

The American Biology Teacher, Vol. 73, No. 8, pages 469–473. ISSN 0002-7685, electronic ISSN 1938-4211.
DOI: 10.1525/abt.2011.73.8.8

- Knowledge of some different types of liquid culture media and how to prepare them
- Information on the biology of *Euglena*
- Application of population growth models to their data set
- Calculation of percent error

In addition, we stress the importance of searching the literature for previous studies related to the students' experimental question. A great deal of research has been done on *Euglena* and other unicellular photosynthetic organisms, so most students can find ample material using standard scientific databases available to most university libraries. Even Google Scholar turns up some useful papers.

Euglena are readily available from biological supply houses and can be cultured indefinitely in house. For the purposes of student projects, it's important that they are grown in media that provide strong growth in control cultures and that are simple for students to prepare and manipulate. We tested different media before settling on those recommended here. The biology of *Euglena* makes it a good choice for an educational project. *Euglena gracilis* is a freshwater species that can grow in a fairly wide range of temperatures (Sleigh, 1989). Though photosynthetic, they can also operate as heterotrophs, particularly when grown without light (Sleigh, 1989; Walne & Kivic, 1990). The source of organic carbon used by heterotrophic *E. gracilis* includes ethanol, acetate, carbohydrate, and amino acids (Sleigh, 1989, 2000). *Euglena gracilis* can use ammonium, nitrate, nitrite, and amino acids as nitrogen sources (Sleigh, 1989). They cannot synthesize vitamin B_{12} and therefore require it in their medium (Robbins et al., 1950; Sleigh, 1989, 2000). *Euglena gracilis* reproduce asexually. Thus, students can explore questions related to fundamental concepts in cell biology such as autotrophy and heterotrophy, nitrogen, vitamin or mineral nutrition, and physical environmental conditions.

○ Materials

- *Euglena gracilis* stock cultures from which students will inoculate their own cultures
- Stock defined medium: Powdered Murashige and Skoog (1962) mineral medium for plant cell culture (MSM medium), available from Carolina Biological Supply and Sigma-Aldrich. Prepare stock solution (1X) according to directions on package. Students will use 0.1X MSM for their cultures.
- Modified defined medium (sMSM): after preparing their 0.1X MSM, students may wish to add 0.5 g sucrose per liter. This provides more rapid initial growth, which may be desired to reduce the time required for the experiment.
- Dried peas to prepare an undefined medium
- Erlenmeyer flasks, 50 or 125 mL
- Micropipetters and tips capable of pipetting in the 1000, 100, and 10 µL ranges
- Microwave or hotplates for heating water to boiling point
- Aluminum foil
- 3.7% formalin
- 5- and 10-mL sterile glass pipettes and pumps
- Hemocytometers and cover slips
- Test tubes
- Microscopes
- Hand counters (clicker type)
- Space for 10 to 20+ flasks per student group, in lighted conditions, and at normal room temperature
- Additional chemicals and materials as determined by individual student investigation

○ Preparation of Stock Cultures

We obtained our original *E. gracilis* from Ward's Natural Science, but *Euglena* can be obtained from a variety of biological supply companies. They will probably not arrive in the dense cultures most suitable for inoculating many culture flasks, so we recommend establishing multiple cultures in advance of the desired start date and allowing them to grow until the cultures are medium-dark green. For long-term maintenance of stock cultures, we found a simple pea-based medium to be very successful. New cultures can be quickly established every 4–12 weeks to maintain a steady and plentiful supply of *Euglena*. The cultures of *E. gracilis* obtained from biological supply companies are likely a co-culture of *Euglena* and an unknown bacterial complement. Although it is possible to isolate *Euglena* into pure cultures, we do not feel the considerable time involved in this process is justified for the purpose of student projects. As long as reasonable care is taken to avoid introducing additional microbes into the cultures, students should get reliable results.

To prepare the pea medium, add 110 mL of water and 4 split peas to each of the desired number of 125-mL flasks. Place in the microwave and bring just to a boil. Carefully remove from the microwave and immediately cover each flask with an 8 × 8 cm square of aluminum foil and crimp securely over the flask. Allow to cool to room temperature before inoculating with *Euglena*. Use aseptic techniques to avoid introducing other microbes into the stock culture. With a sterile 10-mL pipette remove 10 mL from the center of the stock culture and inoculate up to 4 newly prepared culture vessels with 2.5 mL each. Replace and crimp the foil on each newly inoculated flask. This volume of 2.5 mL assumes that you are working from a robust, medium-green stock culture. If you have a less dense stock culture, adjust the volume, or expect it to take a bit longer for the newly inoculated flasks to be suitable for inoculating a large number of student flasks. Place the culture vessels under desired light, at least the intensity of ordinary room light, at room temperature. Placing them under incandescent bulbs works very well. Our students also grow their cultures successfully in north- or south-facing window light.

The MSM and sMSM media listed above may also be used to establish stock cultures. The pea medium provides the easiest long-term method to maintain *Euglena*. However, establishing some stock cultures in MSM medium in advance of the students' starting date is helpful in avoiding a delay in population growth that often occurs if *Euglena* are transferred from the pea medium into the MSM medium. They will rebound, but the delay might be a problem if time is short.

○ Procedures

A suggested schedule for the project follows.

- First lab day: Students receive instruction in and practice the techniques needed to monitor population growth. They discuss possible projects. This takes a 3-hour lab period.
- Second lab day: Students get final approval for their project and set up their cultures and experimental conditions. This takes a 3-hour lab period.

- Succeeding lab days: Students monitor their *Euglena* cultures once weekly over 4 to 5 weeks of growth. They can do this during regularly scheduled lab time, or outside of lab time, as appropriate.
- Final lab day: Students give oral presentations on their results, and turn in a formal written report.

First Lab Day

If students have not previously used micropipetters, it is helpful to have them practice on a colored material. We have students prepare triplicates of two different dilutions of methylene blue. They place 20 or 40 µL of methylene blue stock solution (30 mg/L) in 1 mL of water in a spectrophotometer tube and read absorbance at 670 nm to test their consistency. We use this opportunity to teach students how to quantify their consistency or lack thereof. Students calculate their maximum (or minimum) percent difference as the largest (or smallest) difference between two absorbances in a series divided by the mean absorbance for that series, times 100. Those who are very careful achieve ≤1% maximum difference, but others may have >25% maximum difference, probably because of improper use of the micropipetters. The latter should repeat the exercise until the results are more consistent. The format of a typical data table is shown in Table 1.

Students then learn how to use hemocytometers to count cells, using *Euglena* from extra culture flasks provided by the instructor. The densities of these cultures are not critical, but the instructor should verify that there are adequate cells for counting. Students remove a few milliliters from the culture of *Euglena* (after gently swirling the flask) and bring it to their table in a test tube. From now on, students must be aware that they need to thoroughly mix their sample of *Euglena* before pipetting from it. They pipet 1 mL of the stock into a new tube, and 0.5 mL of stock plus 0.5 mL of water into a second tube. This provides them with a 1.0× and 0.5× dilution of the stock culture to count. Not only does this give students more experience with pipetting and the concept of dilutions, but it prepares them for the possibility that they may need to dilute their samples in the future to be able to count cells. To get the most accurate counts, students use formalin to kill and preserve the *Euglena*. Into each of two new tubes, they pipet 0.1 mL of formalin, followed by 0.9 mL of the sample (1.0× or 0.5×) they wish to count. These counting tubes may be set up in duplicate or triplicate if desired. Immediately prior to counting, the killed cells must be mixed vigorously before loading the hemocytometer. Students load 10 µL of the sample under the cover slip of the hemocytometer. The procedure for counting white blood cells (count cells in 4 larger squares at the corners of grid, each containing 16 small squares) generally works best for counting *Euglena*.

Table 1. Students enter absorbances of two dilutions of methylene blue solution at 670 nm in this table.

	Series A	Series B
Absorbance 1		
Absorbance 2		
Absorbance 3		
Mean absorbance		
Max % difference		
Min % difference		

Once students have counted cells in the two dilutions, we instruct them to repeat the process at least two more times. They then calculate the raw cell density (total cells counted in 4 larger squares/0.4 µL volume counted = cells per µL) for each trial. This is not the final density because 0.1 mL of formalin was added to the sample prior to counting. We use this opportunity to introduce the standard formula for dilutions: $C_iV_i = C_fV_f$, where C_i = the initial concentration of cells in the sample (unknown cell density to be calculated), V_i = the initial volume removed from the sample (0.9 mL of culture used), C_f = the final concentration of cells in the diluted sample (the raw cell density calculated from hemocytometer counts), and V_f = the final volume of the diluted sample (1.0 mL, since 0.1 mL formalin was combined with 0.9 mL of culture). Rearranged to solve for the initial concentration in the sample, the equation becomes $C_i = (C_fV_f)/V_i$. When students perform this calculation, they see that the actual cell density in the sample is greater than what they counted on the hemocytometer because of the added formalin. The ratio V_f/V_i corrects for this. This is a calculation that they must apply to every count they make in the future. Students then compare the results between the two dilutions to see that they must make an additional correction for the 0.5× dilution. They also compare the replicate results within each dilution and again calculate percent difference. The format of a typical data table is shown in Table 2.

Students then discuss possible projects among themselves and with the instructor. Instructor guidance is critical at this point to help students assess the feasibility and scientific merit of various possibilities. In our experience, students are more likely to propose projects that are too involved and require more work than is reasonable than they are to propose overly simplistic projects. By the end of the first lab day, all groups have verbal approval of the concept of their experimental design. They inform the instructor of any special materials they may need (e.g., light timers, herbicide). We require them to turn in a written proposal, including a detailed list of materials needed, before the next class meeting. They must also develop data sheets tailored to their experiments. Table 2 is a helpful model, but it must be modified considerably to fit the needs of each project. The results of their error analysis help students see the importance of counting repeated samples from each culture. We suggest that they set up five culture flasks per treatment.

Second Lab Day

The instructor returns the written proposals to students and goes over possible changes in design details with each group. Students prepare their chosen media (sMSM, MSM, or pea), inoculate their cultures, and take initial counts according to procedures given above. Nutritional studies require the use of a defined medium (MSM or sMSM), whereas studies on physical conditions or toxins can use defined media or the undefined pea medium. Students then initiate the treatments according to their experimental design. Some examples of past student projects are listed below.

- Do *Euglena* populations grow faster with glucose, sucrose, or acetate as a carbon source? These students assessed their results in light of the biochemical pathways of cellular respiration. They discussed the concepts of autotrophy, heterotrophy, and osmotrophy.
- How do different concentrations of sodium chloride affect population growth? These students linked their results to concerns about fresh water becoming salinized from sea-water infiltration

Table 2. A table for recording raw counts, estimated population density, and error estimates for samples of two dilutions of a Euglena culture.

	1.0 (stock)					0.5 dilution				
	1	2	3	4	T	1	2	3	4	T
Trial 1 count										
Trial 2 count										
Trial 3 count										
Raw trial 1 density (cells μL^{-1})										
Raw trial 2 density (cells μL^{-1})										
Raw trial 3 density (cells μL^{-1})										
Corrected trial 1 density (cells μL^{-1})										
Corrected trial 2 density (cells μL^{-1})										
Corrected trial 3 density (cells μL^{-1})										
Mean density (cells μL^{-1})										
Max % difference										
Min % difference										

and agricultural practices. They applied the concepts of osmosis and diffusion in their explanations.

- Does additional nitrogen increase the population growth rate? These students discussed their results in terms of nitrogen's role in cell biology, the concept of limiting factors, and cultural eutrophication.
- Do populations grown under a photoperiod of 12 consecutive hours of light and dark grow at a faster rate than those whose 12 hours of light is divided into six 2-hour periods? These students assessed their results in light of research on biorhythms and photosynthetic activity.

In the examples above, students monitored population growth, as expected, since we provide them with the tools to do so. However, some creative students have chosen to investigate other responses of *Euglena*. One group monitored the light reaction in treated *Euglena* using the DCPIP method they had learned in a previous lab. Another monitored motility of herbicide-treated *Euglena* using published methods for spermatozoa that involved only a hemocytometer and stopwatch.

Nutritional studies that require removal of a particular nutrient are not easily done using the MSM medium. However, the students or instructor could prepare solutions of the individual components of the MSM medium, and use them in the desired combinations.

Succeeding Lab Days

Students monitor their cultures, usually by counting samples once per week. They improve in both efficiency and consistency over time. We use lab periods for other exercises for the remainder of the term, so students use different approaches to complete their project. Some groups come to lab early or stay later to count their *Euglena*, others come in on a different day, and still others are able to count within the time constraints of the lab period. We receive no complaints about the "extra" work. Indeed, most groups seem genuinely interested in the progress of "their" *Euglena* populations.

○ Student Assessment, Data Analysis, & Final Reports

With few exceptions, all groups obtain interpretable data. Most commonly, students present their final results by graphing mean population density over time (Figure 1). At the instructor's discretion, they may include standard-deviation error bars or perform t-tests or other statistical tests. We require them to prepare a written report in the format of a scientific journal article, complete with primary literature review. They also give oral presentations on the final day of lab.

We use this project to reinforce some aspects of population ecology, so we require students to calculate an estimate of per capita growth rate (r) for each time interval, using the overall means from each treatment. They then judge whether r is constant, reflecting exponential growth; declining, suggesting logistic growth; or increasing, indicating faster-than-exponential growth. However, this part of the analysis is not necessary for students to be able to interpret their data and can be included or not, as appropriate for the class.

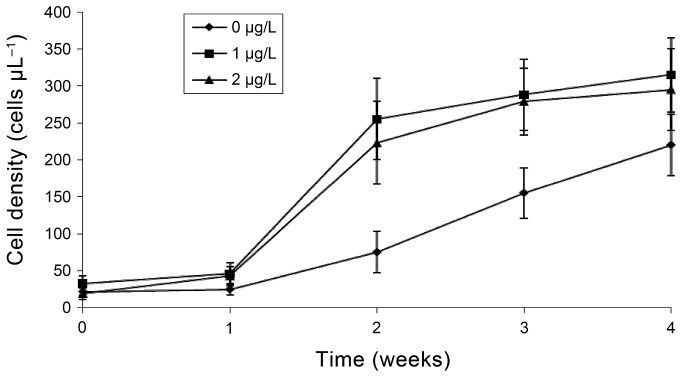

Figure 1. Sample data from student project on the effect of added vitamin B_{12} on population growth in *Euglena*. Means and standard deviations are shown.

○ Conclusion

Most students display considerable enthusiasm when designing and conducting their experiments on *Euglena*, frequently choosing topics related to ecological concerns. Using readily available laboratory supplies and easily grown cultures of *Euglena*, students gain experience in the process of science, a variety of lab skills, and data analysis. They draw on their prior knowledge of cell and population biology and discover new knowledge for themselves through their experiments and literature searches. They perform repeated calculations during data collection and as part of their data analysis. In conversations with their instructors, students frequently commented that they enjoyed conducting an investigation of their own design and were willing to invest greater effort in this project compared to projects that had been assigned to them in previous classes. Many commented that now that they had done the experiment once, they saw numerous ways in which it could be improved or expanded. We have used this project for three terms and found it to be a valuable teaching tool.

References

Ahmed, H. & Häder, D.-P. (2010). Rapid ecotoxicological bioassay of nickel and cadmium using motility and photosynthetic parameters of *Euglena gracilis*. *Environmental and Experimental Botany*, *69*, 68–75.

Danilov, R.A. & Ekelund, N.G.A. (2001). Using the green flagellate *Euglena gracilis* Klebs as physiological dosimeter: is a long-term bioassay more significant than a short-term one? *Turkish Journal of Botany*, *25*, 43–44.

Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*, 473–497.

National Research Council. (1996). *National Science Education Standards*. Washington, D.C.: National Academy Press.

Robbins, W.J., Hervey, A. & Stebbins, M.E. (1950). Studies on *Euglena* and vitamin B_{12}. *Bulletin of the Torrey Botanical Club*, 77, 423–441.

Sleigh, M.A. (1989). *Protozoa and Other Protists*. London, UK: Edward Arnold.

Sleigh, M.A. (2000). Trophic strategies. In B.S.C. Leadbeater & J.C. Green (Eds.), *The Flagellates: Unity, Diversity and Evolution* (pp. 147–165). London, UK: Taylor and Francis.

Streb, C., Richter, P., Ntefidou, M., Lebert, M. & Häder, D.-P. (2002). ECOTOX – biomonitoring based on real time movement analysis of unicellular organisms. *Journal of Gravitational Physiology*, *9*, 345–346.

Walne, P.L. & Kivic, P.A. (1990). Phylum Euglenida. In L. Margulis, J.O. Corliss, M. Melkonian & D.J. Chapman (Eds.), *Handbook of Protoctista*, pp. 270–287. Boston, MA: Jones and Bartlett.

CHRISTINE OSWALD (oswald@sou.edu) is a Professor and STEPHEN KWIATKOWSKI (kwiatkowski@sou.edu) is a Science Laboratory Preparator in the Biology Department at Southern Oregon University, 1250 Siskiyou Blvd., Ashland, OR 97520.