

Using Flour Beetles (*Tribolium confusum*) in Population Growth Studies

Sheryl Shanholtzer¹ and Ann S. Lumsden²

¹Georgia Perimeter College, Department of Life and Earth Science, 2101 Womack Rd, Dunwoody GA 30338 USA

²Florida State University, Department of Biological Science, 2057 Cynthia Dr, Tallahassee FL 32303 USA
(Sheryl.Shanholtzer@gpc.edu; lumsden@bio.fsu.edu)

The flour beetle, *Tribolium confusum*, is an excellent experimental subject that allows measurement of population growth patterns and growth potential in one semester. It is easy to culture and count, and has a high growth rate. It thrives in a scoop of dry flour, can be grown in fruit-fly vials, does not fly, and matures in 40 days at 28°C. A semester-long exercise in population growth for non-major's biology labs, determines the effect of food resources on population growth. A condensed lab for environmental science compares growth curves to the theoretical. Students learn how to both graph and analyze data.

Keywords: population growth, *Tribolium*, flour beetles, experimental design, growth curves

Introduction

Two different exercises are presented. The first is used at Florida State University as a long-term lab for all 44 sections of biology students in their non-major's biology laboratories. It serves as a scientific methods lab in that a hypothesis is tested concerning the effect of resources on population growth. Adult beetles are placed in vials with varying amounts of flour, and counted each week for 13 weeks during the semester. Data are kept on computers within the lab. The same set-up can be used to let students design their own population growth experiments using other variables.

The second exercise was developed at Georgia Perimeter College after learning about the *Tribolium* labs from Florida State University. This lab was developed for use

in an environmental science class without a lab component as a demonstration of population growth potential, growth curve generation and interpretation. Students count beetles less frequently (4-5 times during the semester) and graph the change in population size. It is simple enough to do in a classroom, and takes about 30 minutes to setup or take a count. Graphing skills are emphasized.

The time involved for set-up is minimal. Cultures of beetles are maintained in the lab in vented plastic containers containing a mixture of 50g of brewer's yeast and 950 g of unbleached flour or whole wheat flour. Several times a year the flour needs to be sifted and replenished.

Student Outline

Population Growth in Flour Beetles (FSU)

Introduction

The confusing flour beetle (Fig. 1) is an insect pest of flour and other stored grain products. Like many other insects, they go through a complete metamorphosis (Fig. 2). During complete metamorphosis, the immature stages do not resemble the adult stages (for example, caterpillars and butterflies). Adult female beetles can lay up to 400-500 eggs in stored grains. In 3-5 days the eggs hatch into the actively feeding stage called the larva. A larva grows by periodically shedding (molting) its skin (exoskeleton) until it reaches the size of the adult. It then molts into the pupa, which is the resting stage. This stage finally molts into the adult. The adult then matures and within days is able to reproduce by mating and laying eggs.

The flour beetle is a good insect for life cycle observations. All of its life stages are easy to rear and retrieve, and the life cycle is short. The life expectancy is approximately 6 months. By careful observations you will be able to see a number of interesting phenomena for yourself.



Figure 1. Flour (*Tribolium confusum*) beetles.

Objective

To understand and perform the elements of the scientific method by performing a population growth experiment with flour beetles.

Materials

- 1 glass vial (per group)
- Petri dish
- Beetle growth medium (whole wheat flour)
- Tape (to label vials)
- 6 beetles (per vial)
- Marking pen
- Funnels
- Sifters
- Data table
- Data graph
- Calculator

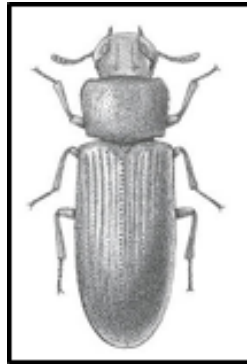
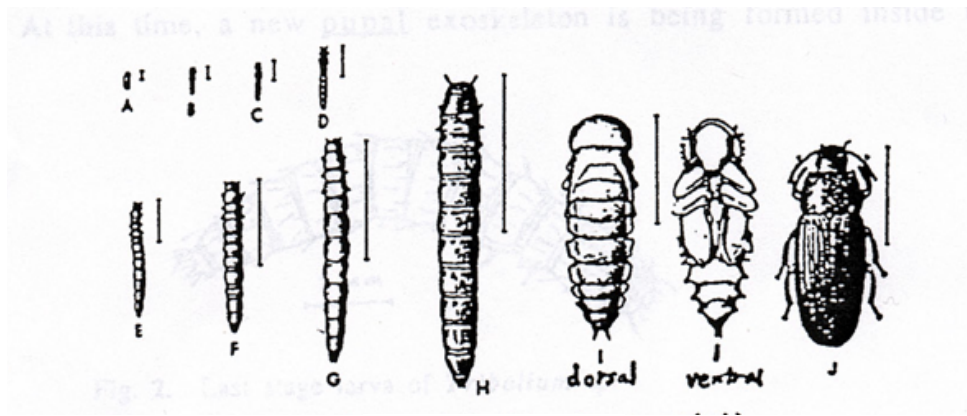


Figure 2. Life stages (complete metamorphosis) of *Tribolium*. A. Egg, B.-H. Larval instars, I. Pupa, J. Adult. (Lumsden, et al., 2010)

Develop a Hypothesis

Observe the populations of flour beetles on display in your lab. Notice that each vial contains a different amount of flour. Develop a hypothesis by noting a relationship between the amount of food resources and population size.

Procedure

1. Select one vial and mark it with a piece of tape with your initials and lab section number written on it.
2. The vials have been pre-filled with 2, 4, 8, or 16 g of medium and 6 beetles have been added to each vial (Fig. 3). Label your vial with the amount of medium.



Figure 3. Vials with flour and six beetles

3. In order to verify the number of beetles, pour the contents of the vial into the sifter. Sift the flour into the Petri dish. After counting the number of adult beetles, return the beetles and the medium to the vial using a funnel (NOTE: Do not leave any flour on the table top. This flour may contain eggs)
4. Stopper the vial with cotton.
5. Make your first entry on your lab data sheet and in the laboratory computers.
6. The lab instructor will put the beetles in an incubator at a constant temperature and complete darkness.
7. Each week count the number of adult beetles in the vial during your lab period, recording the data on your data sheet and in the computer. Again, be careful not to leave flour on the tabletop.

Final Calculations

1. During the last week of observation, make a final population count of the adult beetles in your vial.
2. Your instructor will organize a table of the compiled class data. From this data, calculate the mean growth for each week for each amount of medium.
3. Transfer this information into your data table.
4. Plot the information on a data graph.
5. Determine if your hypothesis has been supported or falsified.
6. Once all the information has been gathered, prepare a final lab report, including the population growth data sheet, the compiled data table and a data graph. By performing this population growth experiment, you have completed the investigative process of making an observation, constructing a formal hypothesis, gathering data, analyzing the data, presenting the data in an understandable fashion, and organizing the data into a conclusion based on the hypothesis.

Guidelines for Writing a Lab Report

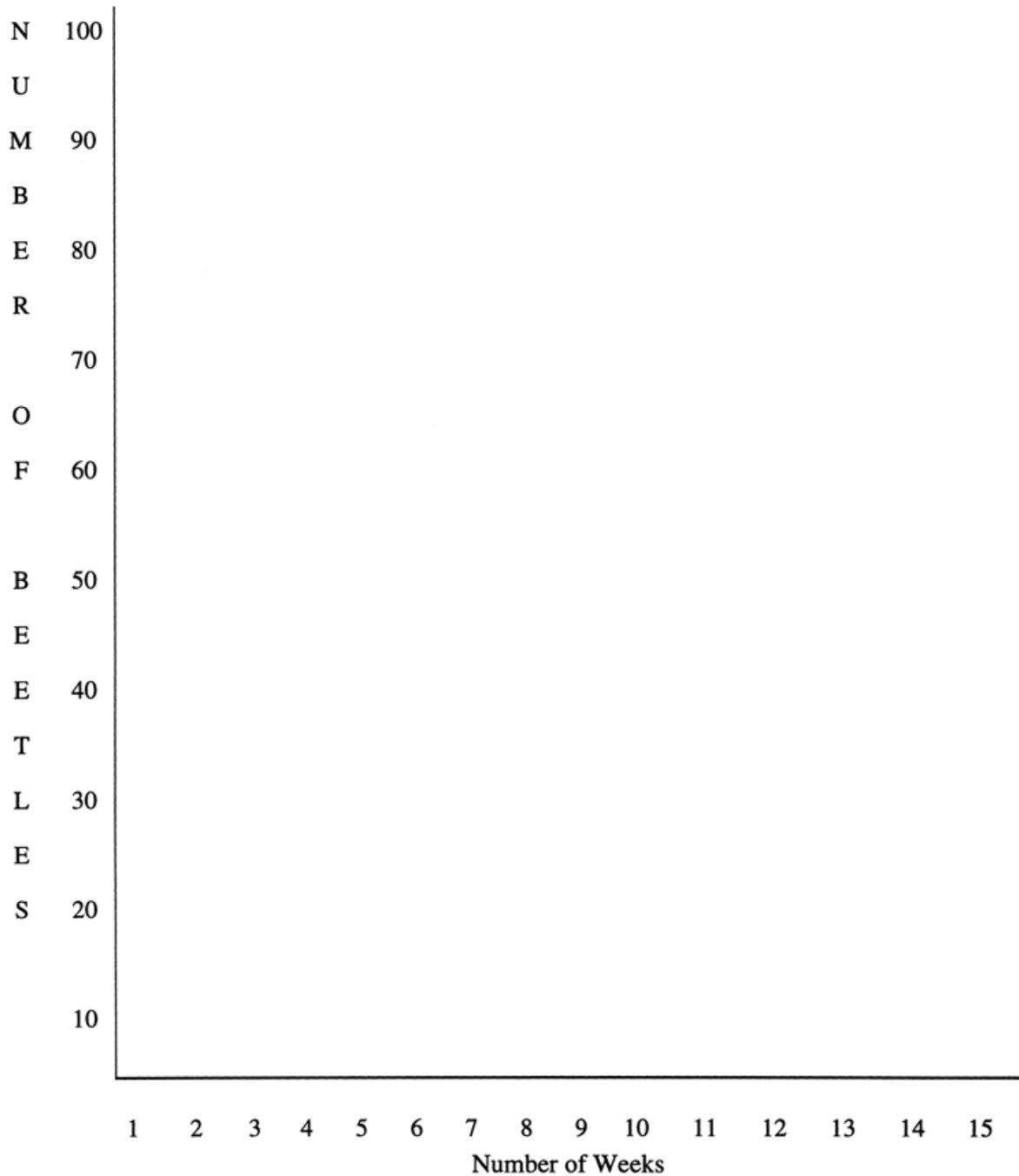
The beetle report must be completed **individually**. All students will be using much of the same data, but you must write your own reports. Feel free to discuss the data and results with your classmates, but **write your own reports in your own words**.

In general, your lab reports should include a minimum of two typed pages, plus data tables and graphs. The reports must be typed, single spaced, 12 pt font, with standard 1 inch margins, and formatted as shown on the following pages.

- **Data Tables**
 - Tables of the overall data for all classes will be provided for you. In addition to the overall data, you are responsible for collecting and keeping the data for your beetles. All of the data, overall and personal, should be presented in table format and included with your report.
- **Graphs**
 - You are responsible for generating graphs that serve to illustrate trends in the beetle data. Generally, line graphs are most appropriate for this type of data. The graph should contain five data lines, four representing the overall class data (provided) and one line representing your individual data (kept by you).
- **A Word about Plagiarism**
 - The use of someone else's work or ideas without proper citation or credit is plagiarism. Therefore copying someone's lab report, either whole or in part, or copying information from a book or the Internet without citing your sources is considered plagiarism.

TRIBOLIUM POPULATION GROWTH DATA GRAPH

Average number of *T. castaneum* in 2,4,8, and 16 grams of flour.



TRIBOLIUM POPULATION GROWTH COMPILED DATA TABLEAverage number of *T. castaneum* reared in 2, 4, 8, and 16 grams of medium.

<i>Week</i>	<i>Date</i>	<i>Population Size at:</i>	<i>2 gm</i>	<i>4 gm</i>	<i>8 gm</i>	<i>16 gm</i>
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						

Guidelines for Writing a Lab Report, cont..

- **Format Instructions**

- Name _____
- Title
 - * Your title should be one sentence (or less).
 - * A reader should be able to tell what the entire report is about by reading the title.
- Introduction
 - * The introduction should include brief background information about the experiment, organism(s) used in the experiment, the objective of experiment, and your hypothesis (your prediction of what would happen before beginning the experiment).
- Materials & Methods
 - * This section describes the materials and procedures used in the experiment. This section must be written out in paragraph form. **NOT AS A LIST!** Explain what you did to obtain the data, the experimental setup, etc. Another student should be able to repeat the entire experiment by reading this section.
- Results
 - * What do the data collected in the experiment show (overall and individual)? What happened in the experiment? Summarize overall trends in the data and explain what the data tables and graphs represent. In addition to written results you must **include the experimental data** (in the form of a data table), and **include line graphs of the data**.

- Discussion
 - * Discuss your results and explain why/how they support or discount your hypothesis. What can you conclude from your results? How do the overall results compare with your individual results? What are possible sources of error in the experiment? Discuss any problems that occurred and ways to fix the problem in the future. How could the experiment be improved?
- References
 - * This section should include references to any works cited in your report. References should also be cited within the text of your report. Any widely used reference format is acceptable (APA, MLA, etc.) as long as it is correct. At minimum, your references would include this lab manual.

POPULATION GROWTH STUDY OF FLOUR BEETLES (GPC)

Introduction

When populations of organisms are allowed to grow under constant conditions, the increase in numbers follows a predictable pattern. A type of graph called a population growth curve is used to describe these patterns by showing the number of organisms on the y-axis (vertical) and the time (or number of generations on the x-axis (horizontal)). These curves are usually S-shaped like Fig. 1 because the numbers of organisms start low, increase ever faster for awhile and then eventually reach an upper limit beyond which they do not increase.

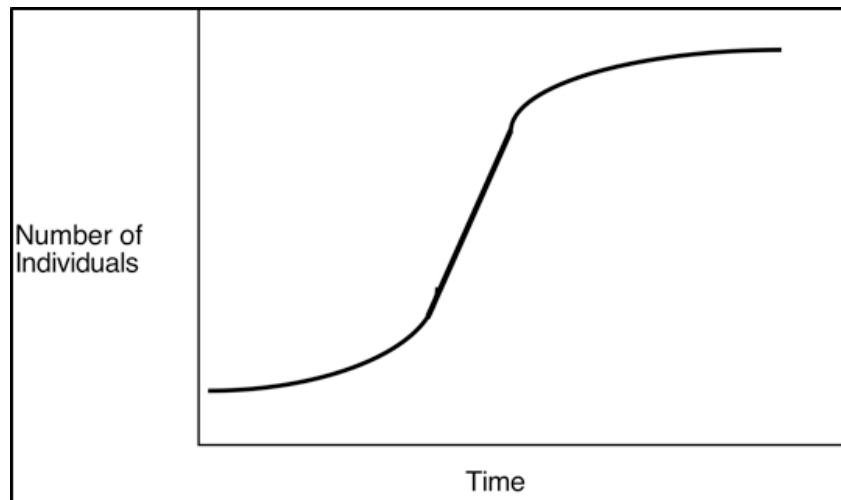


Figure 1. The growth curve of a simple population

The shape of an S-shaped population growth curve is the result of two processes. When the population is small relative to its resources, each individual organism is very successful at producing offspring, and the population grows each generation. If each individual produces three offspring, the population will triple in each generation. When this occurs, population growth is said to be geometric, that is, it increases by a certain factor (by a factor of 3 in this example) in each generation. If population growth continued in this geometric mode, the growth curve would resemble the upper line in Fig. 2, and theoretically, would eventually reach infinity.

In real life however, this can never happen. As population size increases, a wide variety of negative influences will begin to act either on the reproducing individuals or their offspring that decreases their reproductive success. Each adult will produce fewer offspring. Finally when individuals produce, on average, only a single offspring, the population increase will stop. There are many factors that have a negative effect on reproductive success, and they may act on either survival or reproduction. Examples of such factors include space, nest sites, food, disease, shelter, predation, accumulation of wastes, or migration. These negative influences on reproduction and survival gradually put the brakes on population growth until it finally ceases and the population size reaches an upper limit (Fig. 2).

The upper limit of population size is called the *carrying capacity* (K) for that population. The carrying capacity is a characteristic determined by both the organism and the environment. Carrying capacity may be determined by the same factors that slowed the rate of growth - availability of food, shelter, nesting sites, or space, disease, etc. As these environmental conditions change, the carrying capacity of an environment may also change.

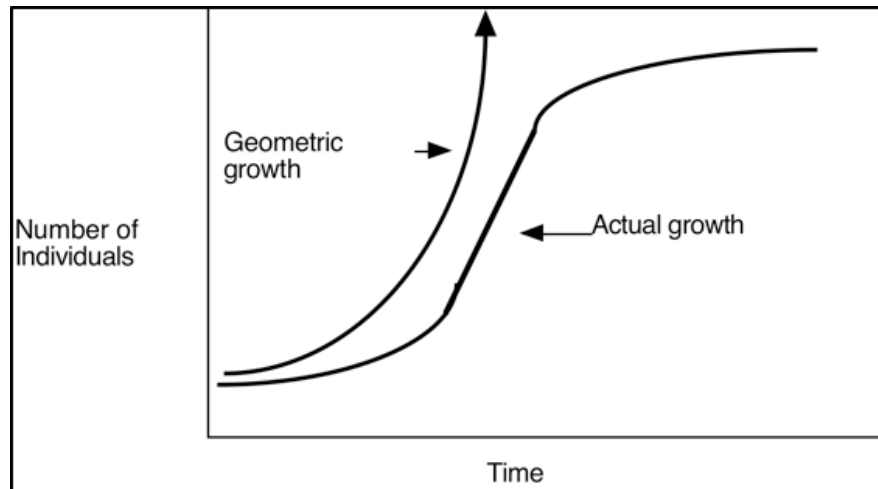


Figure 2. Geometric and actual growth of a population

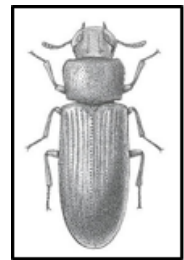
Another way of looking at populations and population growth is from the point of view of the processes that result in changes of population size. There are four such processes: 1) birth, 2) death, 3) emigration and 4) immigration. Births and immigration represent gains in the population, while deaths and emigration represent losses. When:

$$\text{Birth Rate} + \text{Immigration Rate} = \text{Death Rate} + \text{Emigration Rate}$$

the population size will be stable, neither increasing nor decreasing. There are many factors that influence these four factors.

The nature of the factors that result in population growth and stabilization are central questions in ecology, and have been the subject of a great deal of research. In real life, populations of species generally interact not only with members of their population but with other species as well as their physical environment. This makes understanding and predicting population growth in natural populations very complex and difficult undertaking.

We are going to demonstrate the principles of population growth by studying it in a simplified system with only one species and a non-renewing environment. To do this we will use **flour beetles**. The flour beetles of the genus *Tribolium* are ideal subjects for this study. They are small, have short life cycles, require little care because they spend their entire life cycle in dry flour, can be easily counted at intervals, and can take the kind of abuse that you can dish out. The vial of flour is their universe - it both supports them and limits them.



Objective

To follow the size of a population of flour beetles established in a vial of flour for about three months. The data will be graphed and the graph compared to theoretical growth curves to determine growth potential, and carrying capacity.

Materials

- Vials with plugs
- Labels
- Paint brushes
- Typing paper
- Beetles (10 per vial)
- Beetle growth medium (whole wheat flour)

Procedure

The growth of flour beetle populations will be studied under the simplest conditions. Each student pair will start a population of flour beetles by adding ten beetles to a vial of fresh flour. By starting with ten we won't have to sex them. Out of ten individuals the chances are about 99.9% that at least one will be female. Because neither immigration nor emigration is possible under these conditions, only births and deaths will affect population growth. Our general procedure is simple: periodically, the flour will be sorted and the adult beetles counted; their numbers entered on the data sheet; and plotted on graph paper.

For our study we will only look at the adult stage (unless you are ambitious and wish to count other stages as well). Adult females reproduce by laying eggs, and the number of eggs she lays per week can be viewed as her initial reproductive success. Her final reproductive success is best measured by how many new adults she produces.

Detailed Instructions

1. Place 1 level scoop of flour into a vial.
2. Place 10 adult flour beetles into the vial and plug.
3. Write your name, instructor’s name, and the date these were started on the vial.
4. Return the vials to your instructor so that they can be placed in a 28°C incubator.
5. At intervals determined by the instructor, dump a portion of the contents of the vial onto a sheet of paper. Using a brush, sort through the flour and count the adult beetles. Put them into a holding vial using a curled sheet of paper, and repeat until all the flour has been sorted. Be sure to check that no insects remain attached to the paper.
6. Record the number of living and dead adults and note the presence of larval and pupal stages.
7. Put all the insects back in the original vial. Mix by tilting and rolling the vial.
8. Fill in Table 1 each time you count.
9. Begin to produce their population growth curve by plotting the data point after each count.

Table 1: Data Sheet for Flour Beetle Population Study

Date	Number of live adults	Number of dead adults	Notes

Instructions for writing Beetle paper

- Introduction
 - Give the purpose of the experiment.
 - State which organism you will use in the study and why it is suitable. Describe briefly why this problem is important to study. State what you predict the outcome of the experiment will be and why you think so (this is your hypothesis).
- Methods
 - Give the exact procedures **you** used.
 - Give dates counted.
- Results
 - Summarize what you found out.
 - Provide a graph showing population numbers during the time of the experiment.
 - Make sure that the units are correctly spaced on the axes.
- Discussion
 - Discuss whether or not the outcome is as predicted.
 - If so, how closely does it match – are there other factors involved?
 - If not why not – what other factors might be involved.
 - What else could you do to improve your study?

Materials

FSU Experiment

Students working in pairs will need these supplies:

- Flour medium –scoop - about 7 g (stock 50 g brewer's yeast + 950 g unbleached flour)
- Funnel
- Sifter made of PVC pipe with screen glued to the bottom (Fig. 4)
- Petri dish
- Label
- 6 beetles

GPC Experiment

The following are needed per pair of students:

- 1 vial with stopper that allow air exchange, such as fruit fly vials
- 1 extra vial
- Flour medium –scoop - about 7 g (stock 50 g brewer's yeast + 950 g unbleached flour)
- Label
- 10 beetles
- Small paint brush
- A sheet of white paper

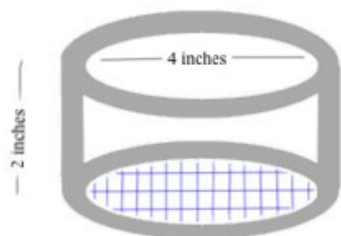


Figure 4. Sifter dimensions

Notes for the Instructor

Culture Notes

The beetles are very hardy and need minimal care. They can be kept in plastic containers with a screen top, such as a 2 quart Glad© food storage container with windows cut in the top and window screen taped over the openings. The stock beetle cultures at Florida State University are maintained on several cups of whole wheat flour while those at Georgia Perimeter College are kept with several cups of stock made from 50 g brewer's yeast plus 950 g unbleached flour. The bins are maintained at room temperature. About every six months, the exoskeletons and dead beetles can be scooped from the top of the flour and new flour added. These have been maintained for over 15 years this way. Starter cultures can be ordered from Carolina Biological Supply.

Care should be taken when counting that surfaces are wiped down afterward so that flour and eggs are not left behind. Excess flour removed when cleaning stocks is placed in a freezer for several weeks before disposal to kill larvae.

General Teaching Notes

These procedures can be used in a variety of exercises, both predetermined or inquiry based. Here we have presented labs, one in which the amount of food varied and one with the same initial amount of food. Of course the culture conditions could be varied as well as the number of beetles. The beetles could possibly be used in toxicology studies.

It is possible to sex the beetles, but for introductory labs we found this too difficult. If this were done, exact numbers of females could be used to start cultures. Park (1934) published an article on the general biology of *Tribolium confusum* that shows drawings of the characteristics of male and female beetles. Visit <http://www.math.unl.edu/~jlogan1/PDFfiles/asdf.pdf> for the paper.

Florida State University Teaching Notes

At Florida State University, this lab is used for the Non-Major Biology Program with non-major students. FSU biology is a large program with 42 sections of lab per week. There are 24 students per lab and 1 teaching assistant running each lab. We assign a pair of students to each vial. There are 12 vials per section and therefore 3 vials with 2 g of flour, 3 vials with 4 g of flour, 3 vials of 8 g of flour and 3 vials of 16 g of flour. Each set of 12 vials is stored in an incubator in the lab room at a temperature around 27°C (80°F). We have glued film canisters to a piece of plexiglass about 4 ½ inch by 11 inch and sit the vials in the canisters. They will be labeled by section and students' names on each of the 12 vials.

Each week, students count the number of adult beetles in their vial. They record that number on a computer in the room for their name and section number and the amount of flour in their vial. At the end of each week, data are collected from the computers in both of our lab rooms and totaled for the 1050 students in labs. At the end of the semester, the number of beetles by amount of flour is totaled so that graphs can be drawn by the students to compare their individual data, the section data, and the entire program of 1050 students' data. Therefore, the students will see that a larger sample size gives better results of data collection than a single vial.

At Florida State University this lab was begun around 1985 or 1986 and the beetle culture has been maintained since then. We add new flour and clean the cultures about every six months. We never buy beetles and use this species because normally they do not fly. The beetle culture is kept in a prep room. At the end of each semester the vials from all sections of lab are dumped into a large plastic container (about a three gallon container) with new whole-wheat flour. We never dispose of beetles because we do the lab every semester and reuse the beetles. All of the vials are washed and reset for the next semester.

Georgia Perimeter College Teaching Notes

This activity can be performed in a classroom. The instructor needs to bring a stock culture, a container of flour, brushes, white typing paper, labels, and clean vials. After the

vials are labeled, the students fill them with one scoop of flour. On the instructor's table, place several pieces of paper with about a teaspoon of the stock culture media (plus beetles) poured onto it. The beetles will start to move and the students can easily brush 10 into the prepared vials. Excess is returned to the stock container. The vials are collected and placed in an incubator at 28°C. For counting, the student culture vials are returned along with another clean vial, a sheet of paper, and a brush. Small amounts of the flour are poured onto the paper, the adults counted, and all of the flour, adults and pupae placed in the clean vial. When counting is complete, all of the material is returned to the labeled vial.

Time between counts can be every two weeks, or can vary. By deliberately varying the intervals, a point can be made about setting up graphs. Students should become aware that the environment in the vial is non-sustaining; therefore the population will never reach and maintain itself at a certain carrying capacity. Also, while not obvious within the time frame of the study, you would expect there to be successive cohorts. The beetles can be kept at room temperature, but warmer temperatures speed up development.

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About the Authors

Sheryl Shanholtzer is a graduate of: Columbia College, SC with a BA in biology, Florida State University with a MS in biology, and the University of Georgia with a Ph.D. in zoology. Her graduate work involved vertebrate biology and salt marsh ecological studies. She has been a Professor of Biology at Georgia Perimeter College since 1982, where she teaches Principles of Biology for majors, Anatomy and Physiology, and Environmental Science. She is very interested in the lab experience and is author of the in-house laboratory manual for the introductory biology major's course. In progress, is an environmental science laboratory manual for which she is an editor and author. Sheryl has been an active member of ABLE - since 1988.

Ann S. Lumsden is a graduate of Millsaps College, Jackson, Mississippi with a BS in Biology. Her MS in Biology is from the University of Central Florida and a Ph.D. from Florida State University. She has been a professor of Biology at Florida State University from 1983-2011. She has taught biology, earth science, and marine biology at Valencia Community College, 1972-1975 and at Tallahassee Community College from 1976-2011. Ann wrote the lab manual *From Cells To Salt Marshes* with Pat Hayward and Greg Freed. The lab manual has been used since 1983 at Florida State University in the Non-Major Biology Program. Ann has been an active member of ABLE – since 1994 and hosted the ABLE Conference at Florida State University in 1998. She served on the ABLE board for about 7 years. Ann retired from Florida State University on Feb. 28, 2011.

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Citing This Article

Shanholtzer, S., and Lumsden, A.S. 2012. Using Flour Beetles (*Tribolium confusum*) in Population Growth Studies. *Tested Studies for Laboratory Teaching*, Volume 33 (K. McMahon, Editor). Proceedings of the 33rd Conference of the Association for Biology Laboratory Education (ABLE), 390 pages. <http://www.ableweb.org/volumes/vol-33/?art=14>

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