Inquiring Minds Want to Know: Introducing Freshmen to Experimental Design

Cynthia A. Surmacz

Department of Biological and Allied Health Sciences
Bloomsburg University
Bloomsburg, PA 17815
Phone: 570-389-4132
e-mail: *surmacz@bloomu.edu*

Cindy Surmacz earned her doctorate in physiology from The Milton S. Hershey Medical Center, Penn State University. She is a professor in the Department of Biological and Allied Health Sciences at Bloomsburg University, Bloomsburg, PA and teaches anatomy and physiology, cell biology, and introductory biology. This paper is based on a laboratory exercise that is conducted in Concepts In Biology I, an introductory course for majors, at Bloomsburg University.

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Introduction

Advocates of science education reform have encouraged us to replace our traditional "cookbook-type labs" with investigative or inquiry-based lab experiences (Beyond Biology 101, 1996; Fox and Hackerman, 2003). Investigative lab experiences engage students in asking their own questions, in designing and conducting their own experiments, and in analyzing and interpreting their own results. Such experiences model more closely how scientists actually learn about the natural world. This mini-workshop demonstrated lab activities that are used in our large multi-section, freshman biology lab at Bloomsburg University to introduce students to how biologists study life. The process of scientific inquiry and the elements of experimental design are initially introduced in lab through the examination of a literature-based case study. Students then work in groups to design and conduct their own experiments to explore aspects of termite behavior. This allows students to apply their learning from the case study and to engage directly in scientific inquiry. These activities portray biology as a creative and collaborative field of inquiry and not a list of facts to memorize. This same investigative approach is modeled again in subsequent labs and throughout our biology curriculum.

Notes for Instructor

Activity 1: Elements of Experimental Design: Lyme Disease Case Study

A literature-based case study on Lyme Disease is used to introduce students to both the process of scientific inquiry and the essential elements of experimental design. The case study used is based on the paper, "Chain Reactions Linking Acorns to Gypsy Moth Outbreaks and Lyme Disease Risk," by Jones *et al.* that appeared in *Science* in 1998. In our experience, this ecological study of medical significance appeals to students with a broad range of interests and is accessible to an audience with little biology background. An abbreviated version of the case study appears in the following student materials. An expanded version of the case study with images is available as a PDF file at <*http://facstaff.bloomu.edu/Surmacz*> under Concepts in Biology 1, Lab Resources. The case study provides real-life observations and a testable hypothesis from which predictions are made. Students examine the authors' experimental approach to identify the variables (independent, dependent, and standardized) and the components of the procedure (treatment level, control treatment, replicates, and sample size.) The data are then analyzed, conclusions are drawn, and the original hypothesis is evaluated. The case study is interactive and is punctuated with questions for the students to answer in their lab reports. For this activity, students are divided into small discussion groups. The lab report also contains a number of application questions on hypotheses, variables, and controls that build upon the foundation established in the case study.

Activity 2: Investigating Termite Behavior: Designing Your Own Experiment.

In this activity, students will apply what they have learned about experimental design to conduct an original experiment on termite behavior. This is an adaptation of an ABLE favorite, the termite lab by Shanholtzer and Fanning (1990) as modified by Pitkin (1999). (See also: Neel, 2000 and Wood and Staub, 2003.) Termites can be collected locally depending on the locale or purchased from a biological supply company. The students will initially observe how termites follow a design they draw on a piece of paper and will develop several hypotheses. One hypothesis is selected to test experimentally. Possible questions to pursue include: Do the termites follow blue lines better than black or red? Do they follow squiggly or straight lines better? Do they follow ink or pencil lines better? Do they prefer ballpoint or felt-tip pens? The possibilities are endless! Students next design an experiment to test their hypothesis taking into account all of the essential elements introduced in the case study (independent variable, dependent variables, standardized variables, replicates, etc.) Predictions are made based on their hypothesis. It is helpful to have timers available in order to collect quantitative data. They can time how long a termite stays on a particular type of line (e.g., ink vs. pencil) and use these values to make a comparison.

So what should happen and why? We won't tell the students….BUT the termite will be attracted to the Papermate ballpoint pen the best. The ink in this pen contains 2-phenoxyethanol, a substance that resembles the trail (or aggregation) pheromone that termites recognize (Chen et al., 1999). The termites generally follow any pattern made with the Papermate ballpoint pen provided the line is fairly heavy and not too close to other lines. Students can work in groups of two to four.

Student Outline

Introduction

Biology is the science that studies living organisms and their interactions with each other and their environment. Living organisms have the following features: order; sensitivity; ability to use and transform energy; evolutionary adaptation; growth, development, and reproduction; regulation; and homeostasis, the maintenance of a relatively constant internal environment. While living organisms share basic characteristics, they are also very diverse. Living organisms come in a wide variety of shapes and sizes and by some estimates there are well over 5 million different kinds of organisms on earth!

As you might suspect, biologists study life in a variety of different ways. Biologists in our own department may be found cloning kiwis; leading student expeditions to the Amazon rain forest; culturing fungi that inhabit bark; measuring changes in blood cholesterol during exercise; investigating adaptations of echinoderms in Antarctica; slicing brain tissue of hypertensive rats to determine neurotransmitter distribution; collaborating with NASA to measure biological rhythms of insects in space; studying the microbiology of compost; determining how chipmunks and squirrels perceive their environment; determining the signals that guide the development of heart muscle cells; analyzing genetic mutations that affect development; checking water samples for the presence of pollution indicators; assessing smoke effects on seed germination; using molecular tools to determine the population diversity of lizards; and identifying viruses that affect crop plants. WOW!

What do these diverse activities have in common? They all stem from a desire to understand the natural world through the process of science. In fact, the word *science* comes from the Latin word meaning "to know." Science is a way of knowing that uses objective information to construct an understanding of the natural world (Moore, 1993). While there is no rigid, single "**scientific method**," there are common approaches scientists use to study life. Biologists make observations, raise questions, construct hypotheses (possible explanations to explain these observations), conduct experiments to test their hypotheses, interpret results, draw conclusions, and pose further questions. Each new discovery in biology leads to further questions, making scientific inquiry a continuous and self-correcting process. This is an exciting time to begin your study of biology. The answers to some of life's most fundamental questions are now accessible thanks to new techniques and innovative experimental approaches. Biology is not a compilation of facts to memorize. It is a creative, imaginative, intuitive, and social endeavor.

What is the best way to learn how biologists do their work? Experience is the best teacher! In this lab, you will explore the power of the scientific method to learn about living organisms. Activity 1 brings the scientific method to life by examining an actual research project on Lyme Disease. This activity will introduce you to the essential elements of experimental design. In Activity 2 you will have the opportunity to design and conduct an experiment to test one of your own hypotheses. Enjoy!

Activity 1. Elements of Experimental Design: A Case Study

Scientists observe the natural world and pose questions. A possible explanation for a given observation is termed a **hypothesis**. A good hypothesis is testable and is useful only if it can be proven false by presenting evidence from an experiment. We cannot prove a hypothesis is true. A crucial step in the scientific method is to design an experiment that clearly supports or rejects the hypothesis. This is one of the biologist's most challenging and creative tasks. Biologists spend considerable time reading the scientific literature and critiquing other experiments before undertaking their own work. In designing a good experiment, scientists must define the variables, outline a procedure, and determine controls. In Activity 1 we will explore how biologists use the scientific method to answer a question by considering a particular example as a case study.

Objectives

1. To understand the nature of science and the process of scientific inquiry.
2. To apply the elements of the scientific method to a particular case study.
3. To understand the following elements of experimental design: identification of variables (dependent, independent, and standardized); designing the procedure (selection of level of treatment, number of replicates, sample size, and control treatment); and ability to make predictions.

Materials

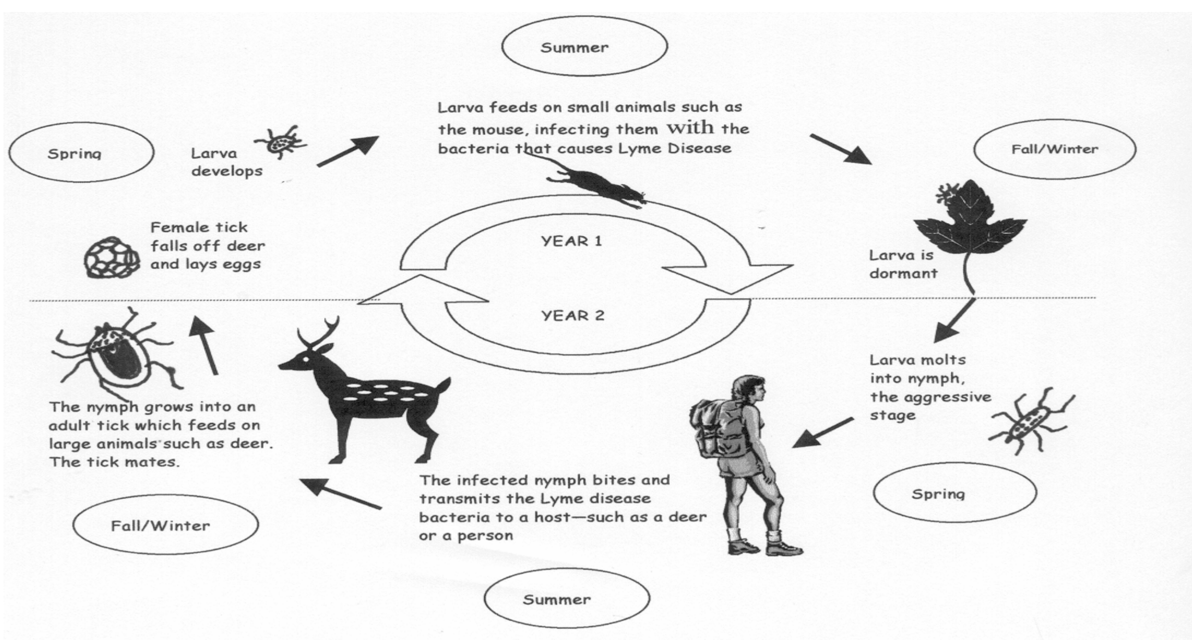
Case Study: *Lyme Disease: A Case Study in Scientific Inquiry* (based on Jones, C., Ostfield, R., Richard, M., Schauber, E., and J. Wolff. 1998. Chain Reactions Linking Acorns to Gypsy Moth Outbreaks and Lyme Disease Risk *Science* 279: 1023-1026 and J. Kaiser 1998. Of Mice and Moths--And Lyme Disease? *Science* 279: 984-985.)

Procedure

1. Read the summary of the case study below BEFORE lab.

Case Study: Lyme Disease

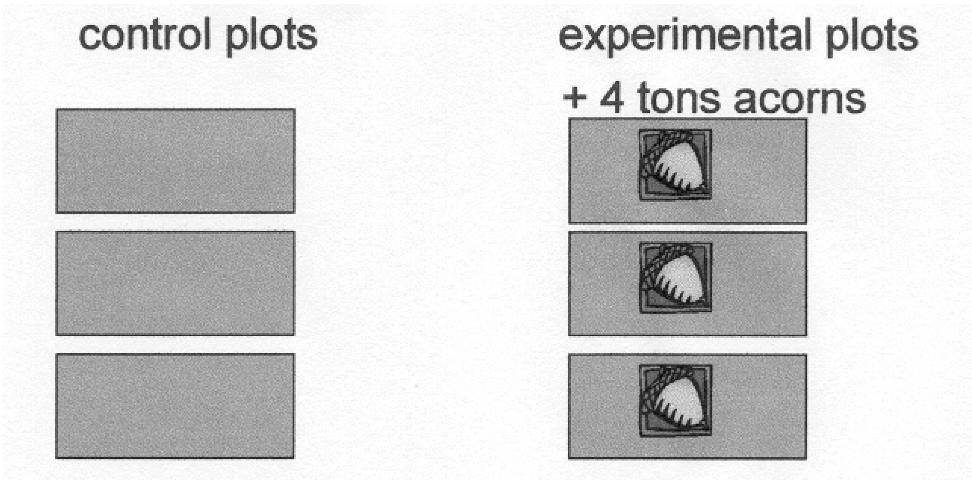
Lyme disease is a prevalent pest-carried disease in the United States. If it is left untreated, it can become debilitating causing heart and nervous system problems and severe arthritis. A spiral shaped bacterium called *Burrelia burgdorferi* causes Lyme disease. The bacterium is transmitted to people by a small, black-legged tick that feeds on deer and mice. A summary of the life cycle of the tick that carries Lyme Disease is shown below:



(From: Lyme Disease, 2000; University of Rhode Island Tick Research Laboratory 2000; and American Lyme Disease Foundation, Inc, 2003.)

The ticks' hosts, the deer and mice, feed on acorns in oak forests primarily in the northeast and the western parts of the United States. A team of researchers observed that the acorn density of the oak forests in New York fluctuates from year to year and that mice populations in the forest were increased when the acorn density was high (Jones, Ostfeld and Wolff, 1996). These researchers asked, "If there are more acorns and mice in the forest, will there be a higher density of ticks that carry Lyme Disease?" They hypothesized that the number of ticks carrying Lyme disease would be high when acorn density in the forest was high.

To test their hypothesis, the researchers studied six unfenced oak forest plots in New York. Mice were initially removed from each plot by trapping. Four tons of acorns were added to three of the plots (with the help of some local girl scouts!). The remaining three plots did not receive acorns.



Ticks were collected in 225 sections (15m X 30m) in each plot, and were identified and counted. The number of mice in each plot was counted also.

2. Review the elements of experimental design presented in your class discussion by answering the following questions in your lab report:
   What is the key OBSERVATION related to this experiment?
   What is the HYPOTHESIS being tested?
   What PREDICTION can be made from the hypothesis? Phrase it in the form of an if/then statement.
3. *Determining the variables*
   When designing an experiment to test a hypothesis, it is essential to identify and carefully consider the **variables**. Variables are the factors that may change during an experiment. The variables must be clearly defined and measurable. The table below describes the types of variables to consider when designing an experiment.

TYPE OF VARIABLE	DESCRIPTION
DEPENDENT	This is the variable that the researcher actually measures, counts, or observes. The DEPENDENT variable is what the researcher thinks will change in response to the experimental treatment.
INDEPENDENT	This is the variable that is intentionally changed by the researcher. An INDEPENDENT variable is selected that the scientist thinks will affect the dependent variable.
STANDARDIZED or CONTROLLED	These variables are held constant between each group. By keeping STANDARDIZED (or CONTROLLED) variables equal, this helps to prevent these factors from influencing the dependent variable.

In this case study, WHAT ARE THE DEPENDENT, INDEPENDENT AND STANDARDIZED VARIABLES? Record your answers in the lab report. DISTINGUISH between dependent and independent variables in the examples in your lab report.

4. *Designing the Procedure*

The **procedure** refers to the actual method or particular series of steps used to conduct the experiment. When designing the procedure it is important to consider the control treatment, the level of treatment, the number of replications, and the sample size. Components of the procedure are outlined in the table below.

COMPONENT OF PROCEDURE	DESCRIPTION
LEVEL OF TREATMENT	The value of the independent variable.
REPLICATION	The number of times the experiment is repeated.
SAMPLE SIZE	The size of the group or portion of the whole that is being assessed.
CONTROL TREATMENT	A group in which the independent variable is held at zero or at some standard or established level.

In this case study, WHAT IS THE LEVEL OF TREATMENT, THE NUMBER OF REPLICATES, THE SAMPLE SIZE, and THE CONTROL TREATMENT? Record this in your lab report.

5. *Predictions*

Remember that a good hypothesis is testable and can be either supported or proven false. It also can be used to PREDICT the effect of the independent variable on the dependent variable. Predictions can be expressed in the form of if/then statements. (General form: If *the independent variable is X and an experiment is conducted in which X is varied* , then *the dependent variables will be affected in this way.)* Predictions are useful when evaluating the experimental results. If the results do not match your prediction then the hypothesis is false. If the results match, the hypothesis is supported.

PREDICT the results of the case study experiment based on the hypothesis. Phrase your prediction in the if/then format in your lab report.

6. *Check Your Understanding*

To check your understanding of hypotheses, variables, and controls, complete Section B of your lab report.

Activity 2. Investigating Termite Behavior: Designing your Own Experiments

Finally…the ball is in your court! You will have the opportunity to design and conduct your own experiment to test a hypothesis relating to termite behavior.

Termite Background

Termites are small, light-colored, soft-bodied insects that live in moist, dark environments. Termites eat wood and can cause considerable destruction to various wooden structures. Their ability to degrade wood and other vegetation provides a nutrient supply for other organisms and makes them significant decomposers in the ecosystem. Did you know that termites do not digest the cellulose of the wood directly? The gut of the termite houses a protozoan that contains cellulase, the key enzyme for digesting cellulose in the ingested wood. This is an example of *symbiosis*, a type of biological relationship where one species lives in or on another. In the specific case of the termite and the protozoan, both organisms benefit from the relationship and this type of symbiosis is termed *mutualism*.

Termites are social insects that live in colonies and have a structured caste system. Most of the labor in the colony is accomplished by the workers. The workers are sterile and lack eyes. Their primary tasks are to collect food and to feed the other members of the colony (queen, soldiers and young). They do this by excreting food from their mouths and their anuses.

Resources: Myles (2003) and Neel (2000).

Objectives

1. To use the scientific method to answer a question that you have proposed.
2. To gain experience in designing and conducting an experiment.
3. To analyze, interpret, and share experimental results

Materials

- several sheets of plain white paper, 8.5 in. by 11 in.
- termite experiment kit containing assorted pencils, pens, and markers of different colors and small paint brushes
- live termites (available from a variety of vendors)
- timers

Procedure

Adapted from: Shanholtzer, S. F. and M. E. Fanning, 1990. "Termites and the Scientific Method", Tested Studies for Laboratory Teaching, Vol. 12
Pitkin, R. 1999. Principles of Biology Laboratory Manual. Shippensburg University.

1. *Make Observations*

   Use the paintbrushes to transfer several termites to a sheet of white paper. Treat them gently, taking care not to crush them! Observe the termites as they move about the paper. If any attempt to leave the paper, gently nudge them back onto the paper with your paintbrush! Draw some lines on the paper using some of the pencils and pens from your termite experiment kit. Does this affect termite movement? Feel free to use additional paper if yours become overly cluttered up with lines. Your goal here is to explore, noting any patterns or interesting observations. Do not attempt to collect data or arrive at any particular conclusions.

   RECORD THREE OBSERVATIONS about termite behavior in your lab report.
2. *Develop Hypotheses*

Discuss your observations of termite behavior with your lab partner. Based on your observations, come up with a list of hypotheses about termite movement. For example, you might guess that termites usually travel in straight lines compared to squiggly lines. Remember that a good hypothesis is testable and can be proven false.
RECORD THREE HYPOTHESES in your lab report.

3. *Select a Hypothesis*
   Pick one of your hypotheses to test experimentally, and record your selected hypothesis in your lab report.

4. *Design an Experiment*
   Design an experiment to test your hypothesis. Write a specific, detailed procedure in your lab report. Consider what kind of data can be collected. Timers are available to determine exactly how long the termites exhibit a particular behavior. Be sure to consider the following elements of experimental design and to include these in your lab report: dependent variable, independent variable, standardized variables, number of replicates, level of treatment. Prepare a table or chart to organize your data. Remember that a good experiment can be repeated.

5. *Make a Prediction*
   Make a prediction based on your hypothesis and experimental design. Phrase your prediction in an if/then form and record it in your lab report.

6. *Collect Data*
   Conduct your experiment following the procedure that you developed. Collect data from each test and record it in the table or chart you prepared in the last step. Record the results of all trials--do not eliminate any data!

7. *Analyze Results*
   Examine your data and consider various ways to present the results. Some suggestions for data analysis include: calculating an average for each condition, providing a range for each condition, graphing the results, and preparing a summary chart

8. *Draw Conclusions*
   Based on your analysis of the results, reconsider your original hypothesis and prediction. Do you accept or reject your hypothesis? Explain why in your lab report.

9. *Present Your Research*
   Biologists share their findings with the scientific community. There are many ways to communicate--giving talks or presenting posters at professional meetings, participating in conferences, and writing papers for journals. Over a half-million new research articles are added to the scientific literature each year. Before an article is published, scientists familiar with the field carefully evaluate it, a process called **peer review**.
   SHARE THE RESULTS OF YOUR GROUP'S TERMITE BEHAVIOR EXPERIMENT WITH THE CLASS. Are your results consistent with your classmates? What new hypotheses should be tested?

Student Lab Report

Activity 1. Elements of Experimental Design: A Case Study.

A. Identify the following elements in the Lyme disease case study by completing the table:

KEY OBSERVATION	
HYPOTHESIS	
PREDICTION (Phrase as an if/then statement)	
VARIABLES: INDEPENDENT	
DEPENDENT	
STANDARDIZED	
COMPONENTS OF PROCEDURE LEVEL OF TREATMENT	
REPLICATIONS	
SAMPLE SIZE	
CONTROL TREATMENT	

B. CHECK YOUR UNDERSTANDING

1. *Hyping Hypotheses*

Circle each of the following that could be used as **scientific hypotheses.**

a) Cats are happy during a full moon.

b) The ozone layer has decreased in thickness over the past century.

c) Blood cholesterol levels will be lower in people following the Atkins diet.

d) Immoral behavior occurs more frequently in the summer months.

e) Students who read their manual before lab get better grades on lab practicals.

2. NAME THAT VARIABLE! Identify the independent and dependent variables in the examples below:

EXAMPLE	DEPENDENT VARIABLE(S)	INDEPENDENT VARIABLE(S)
a. Your lab instructor's blood sugar is measured daily		
b. The diversity of soil microbes is measured before and after fertilizer application		
c. Light absorption by spinach pigments is measured for red and blue light		
d. Three different brands of mouthwash are tested for their ability to inhibit bacterial growth		

3. UNDER CONTROL! Ima Bionerd is considering the following research topics for an Independent Study project. Suggest an appropriate control treatment for each.

EXAMPLE	CONTROL
a. The effect of caffeine on reaction time in college students	
b. The effect of water pollution on reproductive structures in alligators	
c. The effect of acid rain on rates of photosynthesis in zucchini plants	

Activity 2. Investigating Termite Behavior: Designing your Own Experiments

OBSERVATIONS Record three observations of termite behavior	1.____ 2. _______________________________________ 3. _______________________________________
HYPOTHESES Record three hypotheses related to termite behavior.	1. _______________________________________ _______________________________________ 2. _______________________________________ _______________________________________ 3. _______________________________________ _______________________________________
HYPOTHESIS SELECTED	
PREDICTION Phrase in an if/then Format	
EXPERIMENTAL DESIGN Describe in detail. Include the following: Dependent variable, Independent variable, Standardized variables, Number of replicates, Level of treatment.	DESCRIPTION: Identify variables: Dependent: ________________________________ Independent: ______________________________ Standardized: ______________________________ # of Replicates: ____________________________
DATA COLLECTION Record your results. Construct a table if appropriate.	
ANALYZE DATA	
DRAW CONCLUSIONS Do you accept or reject your hypothesis? WHY?	

References

Beyond Biology 101: The Transformation of Undergraduate Biology Education. 1996. Howard Hughes Medical Institute. Chevy Chase, MD.

Chen, J., Henderson, G., and R.A. Laine. 1997. Isolation and identification of 2-phenoxyethanol from a ballpoint pen ink as a trail-following substance of Copotermes formasanus Shriraki and Reticulitermes sp. Journal of Entomological Science, 33:91-105.

Fox, M. A. and N. Hackerman, Editors. 2003. Pages 37 – 41 and 113 – 114 in Evaluating and Improving Undergraduate Teaching in Science, Technology, Engineering, and Mathematics. Committee on Recognizing, Evaluating, Rewarding, and Developing Excellence in Teaching of Undergraduate Science, Mathematics, Engineering, and Technology, National Research Council. 232 pages.

Jones, C., Ostfield, R., Richard, M., Schauber, E., and J. Wolff. 1998. Chain Reactions Linking Acorns to Gypsy Moth Outbreaks and Lyme Disease Risk. Science, 279:1023-1026.

Kaiser, J. 1998. Of Mice and Moths--And Lyme Disease? Science, 279:984-985.

Lyme Disease. 2000. Retrieved September 23, 2003 from the website of the Center for Disease Control and Prevention, National Center for Infectious Disease, Division of Vector-Borne Infectious Disease, Atlanta Georgia: *www.cdc.gov/ncidod/diseases/lyme/lyme.htm*

Moore, J. 1993. Science as a Way of Knowing. Cambridge, MA: Harvard University Press.

Myles, T. G. 2003. Termites-Urban Entomology Program. Retrieved September 23, 2003 from the website of the Urban Entomology Program, Centre for Urban and Community Studies,University of Toronto: http://www.utoronto.ca/forest/termite/iso1.htm

Neel, C.S. 2000. Termite Trails. Retrieved September 23, 2003 from University of Kentucky, Department of Entomology website: *www.uky.edu/Agriculture/Entomology/ythfacts/resourc/tcherpln/termtrails.htm*

Pitkin, R. 1999. Principles of Biology Laboratory Manual. Shippensburg University.

Shanholtzer, S. F. and M. E. Fanning. 1990. Termites and the Scientific Method, Tested Studies for Laboratory Teaching, Vol. 12.

The Tick Research Laboratory. 2000. Retrieved September 23, 2003 from the website of the University of Rhode Island Tick Research Laboratory: *http://www.riaes.org/resources/ticklab/lyme5.html*

Wood, S. and N. L. Staub. 2002. Doing Science: Testing Termites. Retrieved from the Gonzaga University website: *http://gonzology.gonzaga.edu/digital_portfolios/labs/termitelab.php*

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