Learning the Skills of Research: 
Animal Behavior Exercises in the Laboratory and Field

STUDENT MANUAL

Elizabeth M. Jakob and Margaret Hodge, Editors

Dr. Elizabeth M. Jakob  
Department of Psychology  
Tobin Hall  
University of Massachusetts  
Amherst, Massachusetts 01003  
ejakob@psych.umass.edu

Dr. Margaret Hodge  
The Louisiana School for Math, Science and the Arts  
715 University Parkway  
Natchitoches, LA 71457  
mhodge@lsmsa.edu
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Author Affiliations

Paul Andreadis. Department of Biology, Denison University, Granville, OH 43023.
Mitchell B. Baker. Biology Department, Queens College of CUNY, 65-30 Kissena Boulevard, Flushing, NY 11367.
Daniel T. Blumstein. Department of Ecology and Evolutionary Biology, University of California, 621 Young Drive South, Los Angeles, CA 90095.
Paul E. Brunkow. Department of Biological Sciences, Southern Illinois University Edwardsville, Edwardsville, IL 62026.
Teresa A. Cochran. Department of Biological Sciences, Southern Illinois University Edwardsville, Edwardsville, IL 62026.
Daniel T. Blumstein. Department of Ecology and Evolutionary Biology, University of California, 621 Young Drive South, Los Angeles, CA 90095.
Lisa M. Shauver Goodchild. Department of Biology, University of Akron, Akron, OH 44325.
Marta J. Hersek. Lowell High School, 50 Fr. Morissette Ave., Lowell, MA 01852.
Margaret H. Hodge. The Louisiana School for Math, Science and the Arts, 715 University Parkway, Natchitoches, LA 71457.
Elizabeth M. Jakob. Department of Psychology, University of Massachusetts, Amherst, MA 01003.
Susan W. Margulis. Brookfield Zoo (USA), Department of Conservation Biology, 3300 Golf Road, Brookfield, IL 60513.
Michael R. Maxwell. Department of Biology, University of San Diego, 5998 Alcala Park, San Diego, CA 92110.
Matthew H. Persons. Biology Department, Susquehanna University, Selinsgrove, PA 17870.
Kimberly A. Phillips. Departments of Psychology and Biology, Hiram College, Hiram, OH 44234.
Adam H. Porter. Department of Plant, Soil and Insect Science, University of Massachusetts, Amherst, MA 01003.
Larry N. Reinking. Department of Biology, Millersville University, Millersville, PA 17551.
Catherine Hackett Renner. Assistant Vice President for Institutional Research and Planning, 512 North Hall, Mansfield University, Mansfield, PA 16933.
Michael J. Renner. Office of the Provost, Drake University, 2507 University Avenue, Des Moines, IA 50311.
Garrison Smith. Science Department, Kent School, P. O. Box 2006, Kent, CT 06757.
Paul V. Switzer. Dept. of Biological Sciences, Eastern Illinois University, 600 Lincoln Avenue, Charleston, IL 61920.
R. Stimson Wilcox. Biological Sciences Department, Binghamton University, Vestal Parkway East, P. O. Box 6000, Binghamton, NY 13902.

David A. Zegers. Department of Biology, Millersville University, Millersville, PA 17551.
Introduction

Scientists solve puzzles. Science is not about research techniques, although these are necessary skills: rather, the heart and soul of science is framing a hypothesis and testing it. However, it’s difficult for undergraduates to get the opportunity to have the freedom to test their own hypotheses, especially because in many disciplines, the expense and availability of equipment constrain what can be done in a teaching lab. In contrast, animal behavior offers the opportunity to do meaningful research at low cost with little equipment. Many of the examples in your textbook illustrate the fascinating results that can be gained with little but persistence and a notebook. We are scientists partly because we had good experiences in undergraduate laboratories. One of us (EMJ) carried out an independent project in animal behavior in her introductory biology course in her freshman year. Although her conclusions were not earth-shattering or possibly even correct (she attributed the segregation of large and small species of water striders in different areas of a stream to the fact that, when forced to be near each other, the large ones ate the little ones) she had a great time designing and carrying out the project. The other (MH) carried out a project on spider foraging in her undergraduate animal behavior course. This experience was pivotal in her decision to pursue research in animal behavior as a career, and she has been studying spiders ever since.

Thus, carrying out an independent research project can be wonderful fun, and can help you decide whether you want to pursue science as a career. However, it may not be obvious where to start. As you begin to think about a research project, plenty of questions will arise. How can behavior be best observed and quantified? How should your results be analyzed and interpreted? What’s the best way to present your data? The exercises in this manual are meant to help you in recognizing these problems, and to aid you in designing and carrying out your own experiment.

Our goal with this collection of laboratory exercises is to help you learn the skills of research in a step-by-step manner. Your instructor will pick and choose among them (although you may enjoy browsing through them all). The point of many of these exercises is to get you thinking about some aspect of the research process in a new way, so we hope that you will enter into them with an open mind. This manual is organized into two sections. The first section contains exercises focused on developing particular skills in one or more aspects of behavioral research, loosely organized in the order in which you would carry out a project. We begin with the basics of thinking about and watching animal behavior. Then we move on to data collection, followed by experimental design and analysis. Finally, we finish with a section on presenting your data in several different forms. The exercises in Section 1 are specifically geared to preparing you to conduct your own independent research project. In Section 2 are more integrative exercises focused on particular research questions. These will provide you with an opportunity to practice one of more of the skills that you’ve developed.
All these labs have been tested on various groups of students, but of course we are always eager for suggestions for improvements. Please email any helpful suggestions you may have to Dr. Jakob (ejakob@psych.umass.edu), and we’ll be sure to incorporate them and pass them on to other animal behavior instructors. Good luck on developing your skills and in carrying out an independent research project!

—Margaret A. Hodge and Elizabeth M. Jakob
SECTION 1: THE SKILLS OF RESEARCH

Unlike other scientific data you may gather, such as a readout from a piece of equipment or the location of a spot on a gel, it’s not always obvious how to observe and score animal behavior. After all, behavior often comes in the form of a steady stream of activity. How do you convert that stream into data? How do you identify patterns in behavior? The labs in this section introduce you to some of the problems animal behaviorists face, and give you some experience in methods for making behavioral observations more tractable. Exercise 1 may give you surprising insights into how variable our perceptions of behavior can be. Exercise 2 asks you to generate testable hypotheses about an animal you watch. Exercises 3 and 4 take slightly different approaches to the problem of deciding what a unit of behavior is, and will help you overcome the biases in observation uncovered in Exercise 1.
Exercise 1: Chance Favors the Prepared Mind: A Role-Playing Exercise in Observation

Paul Andreadis

Goals
1. To be introduced to the act of observing
2. To examine the types of information collected when observation is unplanned and unstructured
3. To discuss the biases each researcher brings to an observational study
4. To identify the formal elements, conventions, and methods of an observational study that can be found in one’s own unplanned observations

Background
In this laboratory, you will perform a role playing exercise. The purpose of this exercise is to allow you to discover the observational skills you have (and don’t yet have), and what “baggage” you potentially bring to the apparently simple process of observing animal behavior. The key to a productive role-playing session is to suspend disbelief and take the challenge seriously—try to genuinely put yourself in the mindset of the researcher described below. After the role is described to you, respond in just the way that you think a person in that situation would respond. Pretend that the instructor and the other students are not present.

Methods
You should remain in your seats during the exercise. The only materials needed are paper and pencil. The instructor will either read aloud the following role-playing narrative (or a modified one), or show you a brief video.

Six months ago, you first traveled to an uninhabited montane forest of tropical Quasiland. The National Geographic Society and The World Wildlife Fund funded your proposal to search for and study a rare animal, the Highland Enigma (Automaton ersatzicus). The first European explorers of this region heard indigenous tales of a fantastic creature. Scientific knowledge of enigmas has come almost entirely from the study of a small number of skeletal remains. Tantalizing inferences about the behavior and ecology of this species have been gleaned from comparisons of its bones to those of better-known species. Living enigmas have only been seen three times by academic zoologists in the last 200 years. You are determined to be the researcher that puts this species on the scientific map. However, you are also motivated to protect its home. Economic pressures to harvest mineral resources have brought the mining industry further into the Quasiland interior every year. A precious metal find in the adjacent mountain range has convinced you that there is imminent danger of large scale habitat destruction. You feel you must find a compelling reason to preserve these beautiful, primal forests.

Despite 6 months of intensive fieldwork, you have failed to even catch a glimpse of the elusive creatures. You have photographed some tracks in the mud, and have collected a couple of scats for chemical and dietary analysis. Your food supplies are gone, you are physically exhausted, emotionally frustrated, and you suspect that you have contracted
malaria. You should have left for home days ago. But, hope springs eternal, so you have been telling yourself “just one more day.” Now you must leave at the crack of dawn in order to make the day-long trek down the mountain to the river below. If your contact meets you tomorrow morning as arranged, you estimate that you will have time to take his boat to the nearest town, take a bush plane back to civilization, and just catch the latest flight out of Port Lessby that will get you back home in time to present your progress report. Failure to deliver your report on time could be disastrous, as a small army of corporate lawyers that represent various industries will be attending your presentation.

On the day of your departure, you have awakened before dawn in order to get a head start. You have broken camp, packed your gear, and are taking your last look around when a sound catches your attention. You turn. You think you see ... an animal ... moving in the treefall gap a short distance up the mountain. You grope in your backpack for pencil and paper, and dash through the forest. You quickly reach the clearing, and as your eyes adjust to the scene, lo and behold, you see ...

At this point, give your attention to your instructor.

Questions and report instructions

Your instructor will give you a short time to record any thoughts which you haven’t yet written down. You will then have an in-class discussion of what you saw and what you recorded.

For your take-home assignment, you should make a photocopy of your original, unedited “field notes.” You will annotate this copy as part of your assignment. Write up your analysis on a separate sheet, using number/letter symbols to flag the various items in your field notes that are being discussed. Identify as many formal elements of behavioral observation as you can, as described in the assigned readings (Altmann 1974, and selections from Martin and Bateson 1993).

Some areas/elements to look for

• Did you describe the general appearance? Did you really describe the animal you observed, or did you describe some concept of that animal from a previous experience?
• Were your observations qualitative or quantitative, or both? Indicate on your field notes which is which.
• Did you make structural or functional descriptions, or both? Indicate on your field notes which is which. With respect to functional descriptions, where did you derive your sense of function, e.g., is there any anthropomorphizing in your descriptions of behavior?
• What behavioral categories did you define? Which behaviors are states? Which are events? Why?
• If you recorded quantitative data, are your observations examples of latencies, frequencies, or durations? Indicate on your field notes which is which.
• Now that you have done the assigned readings, would you do anything differently if faced with a similar opportunity to observe and record behavior?
**Literature cited**


Exercise 2: Generating Ideas From Observations
Elizabeth M. Jakob

Goals
1. To gain an appreciation for animal observation
2. To understand the logic of generating questions, hypotheses, and predictions about animal behavior

Background
Animal behavior research begins with observation. In this exercise, you will gain what may be your first experience in watching an animal for an extended period of time, and you will think about how you might begin to conduct a research project.

Methods
To begin: find a partner. You and your partner will then locate an animal to observe. It must be nonhuman. It must be a wild animal, not a pet (not even a feral cat or dog). It should not be in captivity or in a zoo. Even in urban areas, there are plenty of animals with interesting behavior, including starlings, crows, house sparrows, and squirrels. In suburban or rural areas, your options will expand. Consider insects or spiders (overgrown fields are great places for these).

Part 1: Observation
Select a place from which to view your subject. Settle yourself in quietly. Don’t alarm or interact with the animal. Watch your subject for a continuous 15 minutes if at all possible. Working independently from your partner, take detailed notes. If your animal moves out of sight, find a new one until you can follow one for the entire time. If your animal interacts with another animal, describe the interaction as best you can, focusing on your individual rather than trying to describe everything that both are doing.

Part 2: Description
After your observation is finished but still working alone, use your notes to write a narrative description (full paragraphs) of what the animal looked like and what it did. Be as specific as you can. Your goal is to provide enough detail so that your classmates can visualize exactly what you saw. Please avoid anthropomorphism (attributing human characteristics to the animal). Do not interpret the animal’s behavior; simply describe it. Include the animal’s species (common name, rather than scientific name, is fine; if you don’t know what the animal is, describe it), location, the time of day you observed it, weather conditions, and anything else that might be affect the animal’s behavior.

At this point, compare your description with your partner’s. Look for the following:

- Differences in how you describe physical characteristics of the animal.
- Disagreement/agreement in how you describe what the animal did.
- Differences in the level of detail you presented.
**Part 3: Generating questions and developing a testable hypothesis**

Now carefully review the logic of scientific procedure as discussed in Chapter 1 of your textbook. You will now follow the same procedure that Niko Tinbergen performed when he first watched beewolves returning to their burrows and when he watched gulls removing broken eggshells from their nests. The first step is to ask questions about what you saw. With your partner, generate two questions about the behavior of your animal. One should be an *ultimate* question that deals with either the historical pathways that led to the behavioral trait, or with the selective processes that shaped the trait. The other should be a *proximate* question about the mechanisms underlying the trait, such as the role of the genes, nervous system, hormones, or muscular system in performing the behavior. These questions may deal with exactly the same behavior or with different behaviors you saw during the observation period. For example, in observations of a goose foraging in a cornfield, you might see the goose repeatedly stop foraging and lift its head. A proximate question about this behavior is “Does the goose lift its head at particular time intervals or only in response to an environmental stimulus?” An ultimate question might be “Why might a goose have a better chance of surviving or reproducing if it possesses a proximate mechanism that causes it to look up periodically?”

Now take one of your two questions and generate a speculative answer, or a working *hypothesis*. This should be a testable hypothesis: that is, it should make predictions that you could then go out and collect data to address. For example, think of the ultimate question posed about the goose. One hypothesis about this behavior is that the goose benefits from lifting its head because it is looking around for potential predators that might attack it. This hypothesis can be tested because it makes predictions: for example, it predicts that if a potential predator appears while the goose has its head up, it is more likely to see it than if it has its head down. One could collect data on lots of geese and see if this is generally true—that is, if you are very lucky to see a number of predation attempts. Think about other predictions that might be easier to test. For example, what might you predict about the behavior of geese at the periphery of a group versus the center? Write out your hypothesis and two testable predictions. Also generate at least one *nontestable hypothesis*. A nontestable hypothesis might be “The goose was looking around because it was thinking about how nice the sun felt on its back.” Maybe so, but we’ll never know!

Finally, for each of your testable predictions, describe how you would test it. Think about the data that you would collect, how many animals you might need to watch, and whether you would conduct an experiment or simply collect more observations on undisturbed animals.

So, for this part of the exercise, you need to generate a proximate and ultimate question, a testable and a nontestable hypothesis about one of these questions, two testable predictions that follow from your testable hypothesis, and an outline of how you would test each of those predictions.

**Part 4: Comparing with other groups**

If your instructor suggests, share your ideas with other groups.
Questions
1. How much did your description of the animal’s behavior differ from that of your partner’s? Why did it differ? If you were to work on a long-term project together, how would you ensure that your observations were more similar?
2. Did some behaviors strike you as more interesting to study than others? How would you go about selecting a research question for an independent project on this animal?
3. For each of your two predictions, answer the following questions. If you conduct the experiment you suggested, and find what you predicted, what can you conclude? Is this proof that your hypothesis is correct? Conversely, if you conduct the experiment you suggested, and do not find what you predicted, what can you conclude? Is this proof that your hypothesis is not true? Explain how you would go about strengthening your conclusions about your hypothesis.

Additional reading
[This reading provides interesting examples of testable and nontestable hypotheses.]
Goals
1. To learn to write operational definitions
2. To learn to make reconnaissance observations and the process of devising and refining a behavior coding system

Background
A pinyon jay flies down, digs around in the deep snow, and comes up with a seed. As an animal behaviorist, interesting questions flood into your mind. Is the jay smelling the seed? Looking for particular features of the habitat, like nearby trees, that in the past have indicated seeds are nearby? Remembering the location of a seed he stored months before? Or just lucky? Often what we are most interested in are the processes that underlie behavior, such as memory, curiosity, or decision-making. However, these cannot be directly observed. What we can actually observe is limited to physical events or actions that occur at certain times or in particular contexts. We use careful operational definitions to describe and quantify what we can observe, and then use these data to make reasonable inferences about the underlying processes that interest us most. For example, suppose we are interested in curiosity. Although we cannot directly see the internal process called curiosity, we can see behavioral evidence of it: an animal that is curious will investigate elements of its environment. We might define investigatory behavior in objective terms, and then record the amount of this behavior that occurs in a particular situation as an index of an animal’s level of curiosity.

This exercise is designed to help you learn how to write operational definitions as you practice developing your observational skills. Clearly defining behaviors is a crucial part of any study: this allows you to be consistent over the course of your study, and tells other researchers exactly what you did. You will watch a single animal to identify an interesting feature of its behavior, and write a clear operational definition. Finally, you will apply your operational definition by watching the animal again and measuring the frequency of this behavior.

Methods

Subject
The subject for this investigation will be a single individual of a nonhuman species of animal. Your instructor may suggest or assign the species to be observed or the specific subjects; if not, you may choose the subject. The individual subject you choose must be reliably identifiable by you, or you must isolate it so that you can be sure you are watching the same subject over time. You must report the scientific name of the species (you may have to look this up) as well as its common name. You must also report the age and sex of your subject if there is any reasonable way to obtain this information. You may attach a photograph of your subject to your final report if you wish. You may also visit a farm or zoo, or catch a bug in a jar; be resourceful.
**Materials**

You will need a watch or clock that can tell time to within 1 second and paper and pencil or pen for taking notes. You will also need a data gathering chart. An example of one type of chart is attached, but its use is optional (it includes space for recording more than one behavior simultaneously); feel free to design your own.

**Procedure**

Get comfortable and spend at least one uninterrupted period of 30 minutes just watching your subject. (Play fair: don’t intentionally choose a time when you know your subject will be asleep.) Look for patterns: for example, are there behaviors that occur reliably when the animal is in particular locations? Jot down anything you think is interesting or noteworthy. Write down any questions that occur to you about the subject’s behavior. Pay particular attention to behaviors that you think you might be able to measure accurately. Take a short break (rest can often renew your attention span).

Select a particular target behavior that seemed interesting. Write a specific operational definition of it. Your operational definition should be so specific that someone you’ve never met could read it, know exactly what to look for, and could use the definition to score the behavior precisely the same way you do. For example:

SCRATCH/GROOM SELF—Washing the face, pulling at ears or vibrissae (whiskers), scratching self with hind legs, or rhythmically moving paws or mouth over various parts of the body will be scored in this category. In addition, body movements resembling “wet dog” shaking will be scored in this category.

Notice that the operational definition is concrete and objective. It does not include terms that infer mental states, intentions, or the function of the behavior being recorded.

Exchange operational definitions with another student in the class. Do you feel confident that you recognize the behavior described by your classmate? If you both observed the same species, did you also see the behavior that your classmate describes? Is there something you would add to his or her definition? After this discussion, and possibly through observing your animal further, refine your definition.

Finally, spend 30 minutes observing the same subject again, with the goal of focusing only on the target behavior. Record the number of times in each minute that your target behavior occurs. If the attached data sheet is helpful, use it; otherwise, invent your own.

**Questions and report instructions**

Describe your observations in a 1–2-page typed narrative. Include the following as part of the narrative:

1. Identify your subject.
2. Report the questions you wrote down during the initial watching session.
3. Give your operational definition of the target behavior.
4. Describe the difference between your initial and final operational definitions.
5. Describe any difficulties or surprises you encountered.
6. The benefits of clearly defining behaviors that we study are clear. However, sometimes focusing on only one or two behaviors can have disadvantages as well. Did you notice any difference in how you went about observing the animal in your two observation periods? Can you think of circumstances in which unstructured observations are preferable to structured ones?

Attach your data recording sheet and turn it in to your instructor by the date specified.

**Further reading**
### Sample data sheet

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**Exercise 4: Constructing an Ethogram: Cricket Behavior**  
R. Stimson Wilcox and Margaret A. Hodge

**Goals**
1. To practice describing and categorizing behavior, and to show that you are already excellent at observing animals
2. To distinguish descriptive observations of behavior from interpretations of the behavior’s function or purpose
3. To learn to ask proximate and ultimate questions about behavior

**Background**
When you begin studying an animal’s behavior, where do you start? Doesn’t it seem sensible to begin by asking a question about the behavior?

If you agree with that, we have to ask you—what gave rise to that question? Don’t you have to observe something first before you can ask a question about it?

If you would become excellent at doing research on animal behavior, there is no substitute for learning to know your animals first, by your own observations. Reading about the behavior in the literature is truly not the same. This cricket exercise is based on learning to observe before you ask questions. Insightful questions stem directly from observations.

Our main goal in studying crickets it to observe them closely and compile a comprehensive list of every distinguishable behavior in the repertoire of the crickets. Part and parcel of doing this is to group the behaviors into categories of related types of behavior, keeping in mind that a purely “descriptive description” of a behavior is not the same as attributing a function or purpose to the same behavior. Yet the categories you will come up with will necessarily be a mixture of descriptive attributes and concomitant functions. This sort of list is called an **ethogram**. For examples, see the meerkat and gibbon ethograms in Exercise 6. Ethograms are not a final research product in themselves, but a step on the way to asking testable questions about animal behavior.

When you begin studying an animal it’s better to begin describing behavior for itself rather than attributing function too soon, since it’s easy to misjudge function through **anthropomorphism**, which is attributing human characteristics to animals (as described in Exercise 2). For example, it’s easy to achieve anthropomorphism by superimposing your own sensory bias when interpreting behavior. Suppose that when stripping its antenna through its mouthparts, as if cleaning it, a cricket produces a sound you cannot hear. Couldn’t you interpret the cricket’s behavior as grooming, rather than sound production? (Actually, this *is* grooming in crickets.) But if the function of antenna-stripping were, in fact, sound production, or both sound production and grooming, you’d misinterpret the cricket’s behavior.

In this exercise, you’ll be constructing a cricket ethogram, by observing them and recording what you see. We know that many people think of crickets as little more than strong contenders with worms for fish bait. But give them a chance. Crickets are pretty amazing animals, with incredibly complex behavior. By the end, we hope you’ll feel that way too.
Methods

**Part I**

Begin by obtaining a container with two males and two females of the common cricket, *Acheta domesticus*. Make sure you can tell males from females; the females are usually larger and have the sword-like, brownish ovipositor protruding from their rears. Make sure all individuals are fully winged—this indicates that they are adults. Take them home with you and amaze your roommates—although said roommates may suggest a different word. Each day or evening during the next week, study your crickets closely, and carefully write down observations on all aspects of their behavior you see. This should include not only actions of individuals but also interactions among individuals as well.

As you progress, construct a list of behaviors, with enough detail so that someone who reads your description can recognize the behavior if they see it. And as you make your list of behaviors, begin to cluster them in what you suspect to be functional categories (such as grooming, sound production, mating, etc.). Note that a category like mating involves interactions between or among crickets. Is sound production also a form of social interaction?

Expect your descriptions and categories to undergo several revisions, and keep in mind that categories can be clustered under other categories, and that you’ll have relatively few really large categories. For example, sound production and mating can be grouped under reproductive behavior, but grooming would go under maintenance behavior. Thus, your ethogram will end up being an interwoven combination of descriptive and functional categories.

Learn to be truly observant. For example, do both males and females make sound? Is only one sound type produced? When and in what specific circumstances are sounds produced? Does sound production involve only one individual? Just how are sounds produced, specifically? What do you think the function(s) of the signal(s) is/are? (And so on.) It should be obvious at this point that detailed observations like these are necessary precursors to asking questions, like these, about functions.

Thus, when you watch and describe the crickets’ behavior, keep asking yourself “What’s going on here?” questions. The idea is to use your powers of observation and curiosity together. Immediately after you begin watching them, it will become obvious that crickets do many things you’ll recognize as similar actions in familiar animals such as cats and dogs—and humans. In fact, you could make a game of trying to find basic things humans do that crickets don’t—setting aside obvious things such as abstract communication, cognition, etc. This is what we mean when we say that you are already excellent observers of animal, or at least human, behavior.

Do you think you would want a separate ethogram for each individual? For each sex? What if you were studying them in smaller or bigger groups, or the wild? Would your descriptions and categories likely change?

You will learn far more if you work with your crickets completely on your own, without reference to categories that your friends may come up with, and especially without reading any literature about crickets. Remember it may be important to know at
what time of day a particular behavior occurred or where the crickets were located (e.g., noisy dorm room versus quiet lounge; or perhaps the doghouse if you and the crickets are banished from your house or dorm). And do you think temperature and other such factors could be important too?

Cricket Care: You might consider marking and naming your crickets. Whether it’s good to name your study organisms has been a point of contention among animal behaviorists—why do you think this is so? If you feel it is appropriate, give your crickets names. If you can’t tell them apart, dab a small bit of white correction fluid or model paint on the top of the thorax. Be sure not to get any fluid on the wings as it will cause abnormal behavior. It becomes apparent immediately that each cricket has a different “personality” from the others. Take good care of them, both for ethical reasons and to make sure you’re seeing normal behavior. Give them water by pouring a little water onto the sand through the hole in the lid. They’ll drink directly from the sand, and if the sand is deep enough (about 2 cm deep), the females will often lay eggs (oviposit) in the damp sand. Add water when the sand tends toward dry. Don’t overdo the water, because if the container gets too wet, the food and feces will mold and rot. Give the crickets the prepared food provided by your instructor, and supplement this if you wish with a piece of apple or carrot about the size of a cricket’s head. Remove uneaten food each day. If any crickets die, replace them from the laboratory stock.

Have fun setting up your system—such as making little cardboard houses for them to dig under and defend, adding rocks and other such things to make their environment more interesting, etc. Try introducing different types of food to see if this elicits new behaviors. And if you want to do an experiment or two on your own, perhaps setting up a system to examine their reactions to different food, light/dark, temperature, etc., go ahead; enjoy being an experimenter. The only thing you’re required to hand in is your ethogram—and the questions mentioned below.

Part II

We freely admit that it’s easy to ask questions about behavior. However, it’s not quite so easy to distinguish between different kinds of questions—aside from whether your question is regarded by your peers as a “good” or “meaningful” question. The textbook distinguishes between proximate causation and questions about behavior and ultimate causation and questions about behavior (as in Exercise 2). Proximate questions address mechanistic causes of behavior that is happening right here and now, performed by an individual or by interactions among individuals before your very eyes. Ultimate questions address evolutionary causes such as how a behavior evolved over many generations, and also address the adaptive value or purpose or function of the behavior. A full analysis of any behavior normally involves answering both proximate and ultimate questions about it.

Given the definitions above and the discussion in first chapter of the textbook, think about the wonderful behavioral patterns you’ve seen during the past week and write three proximate-type questions and three ultimate-type questions. Identify which ones are which.

Next, choose one proximate and one ultimate question of the six you wrote, and
generate at least one plausible hypothesis that derives from each question. Remember that a hypothesis is basically a rendering of the original question into a statement. For example, the question “How do crickets make sound?” may be rendered into a variety of hypotheses about how the sound is made, such as (1) Crickets make sound by scraping their antennae through their jaws, or (2) crickets make sound by blowing air through their spiracles (and so on). Try to make hypotheses that are more plausible than those described above.

Thus, you are responsible for handing in your ethogram, six questions, and two or more hypotheses deriving from two questions.

Further reading
Part B: Collecting Behavioral Data

Now that you have had some experience in looking at animals and thinking about how to describe their behavior, you are ready for an introduction to some standard formal techniques in behavioral data collection. Exercise 5 provides a demonstration of the different information generated by two primary types of behavioral data collection. Exercises 6 and 7 give you a chance to employ these techniques.
Exercise 5: Candid Camera: Comparing and Contrasting Sampling Methods

Susan W. Margulis

Goals
1. To collect data using both instantaneous and continuous sampling
2. To compare and contrast the data that are collected using the two methods
3. To discuss the different uses of the two observation methods

Background
There are two very commonly used methods of collecting behavioral data. In continuous sampling, the observer attempts to record an entire stream of data. Generally, it is possible only to do this for a single animal, and observations on a single animal are called focal observations. Alternatively, one might record what an animal is doing only periodically, at predetermined instants in time (such as every 30 seconds). This technique is called instantaneous or scan sampling, and any behavior that occurs in between the sample periods is ignored. (Both techniques are described in Altmann, 1974). Continuous, focal animal sampling is similar to videotaping an individual for some period of time. Instantaneous sampling is like taking a series of still pictures. Here, you will make this comparison specifically by videotaping and photographing the same animal at the same time and comparing the results.

Methods
Your instructor will select two groups of students (2–4 each) to serve as “recorders” for this experiment. Group 1 will use the camcorder, and group 2 the Polaroid or digital camera. Be sure that recorders are familiar with the operation of the equipment. The remainder of the students will follow the instructions provided by the instructor.

Your instructor has chosen the subject for your observation (these may be animals on the campus or in the classroom, or classmates).

Both groups of recorders will watch the animal (or student) for 10 minutes. The camcorder group records continuously for 10 minutes. The camera group takes one picture every minute (at 1-minute intervals) for 10 minutes. Be sure that both recording groups begin at the same time. The camera group should take their first picture 1 minute after the camcorder group begins videotaping, and their tenth picture when the camcorder group ends their 10 minutes of recording. If you are an observation subject, please behave normally and forget about the cameras. Both recorder groups should focus on the same individual from the same vantage point.

When the recorders have completed the videotaping and photographing, place Polaroid photographs in order in a visible location (for example, tape them to the chalk board or tack them to a bulletin board) or download digital photos to a computer and print them out. Then watch the 10-minute video. Can you identify which photos correspond to which part of the video?

Select one behavior that you observed in the video (locomotion, eating, reading, etc.). Calculate the percent of time that the focal subject spent in that behavior (for
example, if the subject spent 6 minutes and 20 seconds out of 10 minutes eating, then he/she spent 63 percent of the time eating. Next, look at the photos. For each photo, categorize the behavior of the same focal individual. In how many pictures was the subject engaged in the same behavior as you observed in the video? For example, if the subject was eating in 6 of the 10 pictures, then he/she was eating 60 percent of the time.

Questions
1. How similar were the data collected using the two methods? What might explain these differences?
2. What are some types of behaviors that each method might be appropriate for observing? Behaviors can be classified into two types: states, or behaviors of long duration, or events, or behaviors of short duration. Did you see both states and events in your data? Describe them. Which technique is better for measuring states? For events?
3. To summarize the appropriateness of the two observation methods, fill in the following table:

<table>
<thead>
<tr>
<th></th>
<th>Continuous</th>
<th>Instantaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>How many animals can be watched at a time?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good for watching large groups?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good for studying behavioral states?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good for studying behavioral events?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Following are several scenarios of observing. For each scenario, identify which method is better for each situation.
   A. A scientist wants to study how much time zebras in a large herd spend foraging.
   B. A zookeeper wants to find out which male in a group of five monkeys is being aggressive (starting fights).
   C. A scientist wants to know the time budgets of penguins in the zoo so that she can compare them to time budgets of wild penguins.
   D. A new male gorilla has been introduced to a captive gorilla group. A researcher wants to see how he interacts with the other group members.
   E. A scientist at an aquarium is studying how the many fish in a large tank use their habitat. He can distinguish the species, but not the individuals.

Acknowledgments
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Literature Cited
Further Reading
Exercise 6: The Collection of Behavioral Data

Susan W. Margulis

Goals
1. To practice the two most common methods of behavioral data collection
2. To understand the situations for which each method is most appropriate

Background
Now that you’ve learned the basic differences between continuous, focal sampling and instantaneous sampling, you will next have the opportunity to practice these methods. In this lab, we will provide you with two ethograms (see Exercise 5 for more detail about ethograms and how they are constructed). One is for meerkats (*Suricata suricata*) and the other is for white-cheeked gibbons (*Hylobates concolor*). Familiarize yourself with these ethograms.

Methods
Choose a partner to work with. Begin with the video clip of meerkats. Meerkats are a type of mongoose. They inhabit arid areas of southern Africa, and live in groups that usually consist of several families. When foraging, one member of the group can usually be found standing upright scanning the surroundings for danger. This individual is often referred to as a “sentry.”

You will be doing an instantaneous, scan sample of the animals in this group. Carefully review the ethogram and data sheet. The camera will pan (or “scan”) across the group. Watch the tape at least once before you begin your observation.

On your data sheet, tally up how many animals you see engaged in each behavior. Note that you may not always see the same number of animals, as they may run in or out of the range of the camera. You and your partner may alternate roles of observer (watching the tape and calling out what each animal is doing) and recorder (writing the tally marks on the sheet).

Next, move on to the video clip of gibbons. White-cheeked gibbons are lesser apes from southeast Asia. They live in small family groups. Both mother and father care for the offspring. Females are blonde, and males are black. All infants are born with blonde hair, which turns black at about a year of age. Males retain their black hair, while females’ hair becomes blonde again as they approach maturity. The group you will observe consists of a mother and father, and their year-old son.

You will be doing a continuous, focal observation of the infant gibbon. Carefully review the ethogram and data sheet. Watch the tape at least once before you begin your observation.

Use the timer in the upper left corner of the screen to note on your data sheet the time at which a behavior state changes, or an event occurs. Check the appropriate behavior column. Your first entry should always be at time 0:00 (0 minutes and 0 seconds). For those behaviors that involve more than one animal (the focal subject plus another animal) be sure to indicate on your data sheet which animals are involved and
who initiates a behavior. For example, “mom touches baby” is not the same as “baby touches mom.” Be consistent in your use of abbreviations. You may use “m” for mother, “f” for father, and “b” for baby. You may work with your partner or each do an independent observation, and compare your results.

Now look at the sample data on your handout. Compare your observations to the researcher sample. Open the “mock data” table, and use these data to calculate the percent agreement between two observers, following the instructions in the table legend.

Questions
In your write-up, include copies of your data sheets, and those of your partner or a member of another group. Please address the following points:
1. The similarity between observers is called interobserver reliability. How similar were your observations to another student’s observations? How similar were they to the “researcher sample” observations?
2. How do you explain any observed differences? Why is it important to minimize these? What could you do to maximize interobserver reliability?
3. Do you think it is possible to eliminate all interobserver variation? Elaborate.
4. What was the percent agreement calculated from the mock data?
5. What sort of questions about meerkat behavior could be addressed with the type of data that you collected? What about the gibbons?

Acknowledgments
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Further reading
SCAN SAMPLING—Meerkats

Methods

Use scan (instantaneous) sampling to record the behavior of all meerkats that you see at 30-second intervals for 3 minutes. Using the ethogram provided below, enter on your data sheet the number of individuals in each behavior category on each scan. Always begin your scan when the stopwatch beeps. Always start your scan at the same location. You may work in pairs, with one member serving as the “recorder” and one the “observer.” Switch roles mid-way through.

Ethogram

States (for definition, see Exercise 5)
- **Forage**: Animal consumes or manipulates food or digs in the dirt in search of food.
- **Rest**: Animal sits or lies immobile, eyes open or closed, not doing anything else.
- **Play**: Animals engage in rough and tumble social interaction or chasing.
- **Sentry**: Animal stands upright on hind legs, alert, looking at surroundings.
- **Locomote (LOC)**: Animal runs, walks, or climbs around exhibit.
- **Social Groom (SOC.GM)**: Animal uses its teeth or front paws to comb through another animal’s fur.
- **Other**: Any other behavior not listed above.
FOCAL SAMPLING—White-Cheeked Gibbon

Methods
Use continuous focal animal sampling to record the behavior of the baby white-cheeked gibbon. Using the ethogram provided below, observe the youngster for 3 minutes. Whenever he changes his behavioral state, write down the time (minutes and seconds) and check the appropriate behavior column. Whenever he performs an event, write the time and mark in the column who is involved in the behavior: (M)other, (F)ather, (B)aby. For example, if the Father bites the Baby, write “FB” in the BITE/HIT column.

Ethogram

States (for definition, see Exercise 5)
- **Rest**: Animal sits, stands, or lies; immobile; no other activity. Eyes open.
- **Locomotion/Explore (LOC)**: Any movement by the animal, on the ground, rocks, or in trees.
- **Forage**: Animals searches for, manipulates, or consumes food or water.
- **Groom**: One animal runs its fingers or teeth through the fur of another.
- **Play**: Manipulation of objects or apparently purposeless movement. May involve another animal. Note who is involved and describe the play.
- **On Mom**: Infant clings to fur of mother.
- **Other**: Any behavior not listed above.
- **Out of View (OOV)**: Animal is not visible.

Events (For definition, see Exercise 5)
- **Touch**: One animal puts hand on the arm, head, or other body part of another animal. Gesture is gentle and friendly, not aggressive.
- **Bite/Hit**: Aggressive, hard, rapid touch or push, or placing mouth on another animal and closing with apparent force.
Exercise 7: Observing and Quantifying Behavior with JWatcher

Daniel T. Blumstein and Janice C. Daniel

Goals
1. To understand some of the decisions one must make when constructing an ethogram
2. To become familiar with the process of quantifying behavioral observations using an event recorder

Background

Foraging animals must trade-off time allocated to foraging with antipredator (and social) vigilance (read more about this in Bednekoff and Lima 1998). Here, we define vigilance as an animal stopping its activity to lift its head and look about. Because of years of research on this subject in many animal species, we are comfortable in using the functional word “vigilance” as a shorthand for this behavior. We have provided a series of two 2-minute video clips of foraging mammals filmed at Rocky Mt. Biological Laboratory (www.rmbl.org), a subalpine field station near Crested Butte, Colorado. The animals include the following:

1. Squirrel1.mov and squirrel2.mov are golden-mantled ground squirrels (Spermophilus lateralis), a small, asocial, resident ground squirrel. Active during the summer, golden-mantled ground squirrels hibernate throughout the winter. The clips are of two young of the year.
2. Marmot1.mov and marmot2.mov are yellow-bellied marmots (Marmota flaviventris), a mid-sized, social resident ground-dwelling squirrel. Active during the summer, marmots must gain sufficient mass to survive hibernation. The clips are of two young marmots which are fur-dyed as part of a long term behavioral study. The marmots were recorded in early August, a time when they are actively trying to store fat. Clip one is “blot neck,” and clip 2 is “plus back” (the marmot on the left at the start of the video).
3. Hare1.mov and hare2.mov are snowshoe hares (Lepus americanus), a large, asocial, lagomorph. Hares are active throughout the year. In the summer, their fur blends in with the summer vegetation, while in the winter, their pelage turns white—which perfectly matches their snow-covered meadows. Clip one is of an adult hare, while clip two is of a young hare foraging next to a willow thicket.
4. Deer1.mov and deer2.mov are mule deer (Odocoileus hemionus). Mule deer are year-round residents of RMBL. We have clips of two females, one with a young (deer1.mov), and one without a young (deer2.mov).
5. Cow1.mov and cow2.mov are domestic cattle (Bos taurus) which are grazed seasonally in and around the National Forest lands surrounding RMBL. Clips are of a mother (cow1.mov) and her young of the year (cow2.mov).

6. Horse1.mov and horse2.mov are domestic horses (Equus caballus), which are also grazed seasonally. Cattlemen use the horses to herd cattle. Clips are of two males foraging in a temporary paddock.

Methods

Developing an ethogram (10–15 min)

An ethogram is a catalog of behaviors. The first thing you must do when quantifying behavior is to come up with a list of behaviors. We’re going to develop a “partial ethogram” focusing on foraging and antipredator vigilance. Have a look at one of each of the clips. Focus on the foraging and vigilance behavior. Note that some species can forage while simultaneously looking and some species can look while chewing.

Divide into several groups so that several people are looking at one species. Each group should describe, for a species, the motor patterns used for foraging and for vigilance. An example is:

**Horse, Foraging:** Subject stands quadrupedally (on all four legs), head down in the vegetation, clipping and ingesting vegetation with its mouth.

Be sure to include the various postures used while acquiring food and looking (i.e., if you see animals looking while chewing, be sure to define a behavior “looking and chewing”).

Share your resulting ethogram with the other groups and discuss the specificity of your categories and your definitions.

Developing a testable question (5–10 min)

In order to study behavior well, you must have focused questions. Let’s consider antipredator vigilance. A number of obvious questions arise when looking at these different video clips. The one we’re going to ask today is: *Are there differences between the species in the time allocated to foraging and vigilance?* We’re going to examine this question by estimating the time allocated to foraging and vigilance for each of these species. Because we’ve only got two video clips of each species, we will not conduct formal statistical analyses, but rather, we will eyeball the differences in mean time allocation and base our conclusion on this comparison.

Assuming there will be differences, discuss what might explain these differences. For instance, species vary in their domestication, body size, and exposure to predators. While small animals face a variety of predators (e.g., coyotes, foxes, weasels, martens, black bears, hawks and eagles), larger body-sized animals may be relatively safe. Wolves are extinct in most of North America, including at the Rocky Mountain Biological Laboratory where these videos were shot, and black bears do not attack deer-sized
animals. The video clips include animals of different ages and sexes. How might this influence vigilance? How about group size?

Quantifying behavior (45 min)

For the purpose of this exercise, let’s use a simple ethogram that allows us to make comparisons between species.

- \( f \) = head down foraging
- \( r \) = rearing up on two legs while foraging
- \( l \) = standing quadrupedally and looking
- \( c \) = standing quadrupedally and looking while chewing
- \( u \) = standing bipedally and looking while chewing
- \( w \) = walking or other locomotion
- \( x \) = other behavior
- \( o \) = out-of-sight

There are several ways one could estimate the time allocated to foraging and vigilance. We’re going to employ a technique called focal animal sampling, where we focus on a single subject and note what it is doing. When focusing on a single subject, one can time sample or continuously record behavior. Time sampling involves recording what the subject is doing at pre-determined time intervals—say every 1 sec, every 5 sec, etc. Continuous recording is doing just that, noting every behavioral transition (i.e., from foraging to looking) and the time at which it occurs.

There are advantages and disadvantages to each of these recording methods: time sampling necessarily involves missing behavior, but may be less labor intensive. For animals that engage in behaviors that have relatively long durations, time sampling may be appropriate. In contrast, time sampling animals that quickly change behaviors and engage in a number of different activities over a short period of time may lead to inadequate estimates of time allocation. Of course, the shorter the time interval between samples, the more “continuously” you’re recording behavior.

We’re going to employ continuous recording to estimate the time allocated to foraging and vigilance in these mammals, and we’re going to use an “event recorder” to help us. Event recorders are computer programs (or dedicated pieces of hardware) that record keystrokes as they occur over time. In our case, keystrokes will represent behavioral transitions. For instance, when the animal is foraging, you will type an “\( f \),” when the animal is looking, you will type “\( l \),” etc. Using analysis algorithms included in JWatcher, the event recorder, we will then calculate the time allocated to foraging and vigilance. The full JWatcher program and manual is freely available online at: http://www.jwatcher.ucla.edu

Using JWatcher to score behavioral transitions

1. Click on the JWatcher icon to launch JWatcher
2. In the Data Capture tabbed window, name the data file you will be scoring by clicking on the file navigator icon (it looks like a sheet of paper) to the right of the Focal Data window. Choose the location where you wish to save your new file. Type in the name
of your new file in the Filename box and click Open. Keep the names simple. For instance, name the data file for the first ground-squirrel video clip, squirrel1.dat.

3. Specify a Focal Master File (the ethogram along with additional specifications for recording the focal observation by clicking on the file navigator icon). In this case, the focal master file is called lab.fmf.

4. Click the Next button at the bottom right to tab into the next page.

5. Answer the two questions by typing the species and the video clip into the boxes below the questions and click the Next button to advance.

6. Cue up the video clip. There is a 4-second count-down sequence. When ready, click on the Start button. Immediately type the key code representing the behavior the subject is currently engaged in. Whenever the behavior changes, type the key code for the new behavior. Continue for the two full minutes (JWatcher will automatically time out).

Notes and hints:

JWatcher is case sensitive: “f” will not be recorded the same way as “F.” For this exercise, you should use lower case letters.

If you wish to see a list of the behavioral codes on your computer screen, click the Behaviors tab in the upper right corner of the JWatcher screen.

If you made a data entry mistake, discard the resulting data file and start again.

If you are not a good touch typist, you may combine the different types of looking and just type “l,” and the different types of foraging and just type “f.” If you do this, be sure to also type “x” and “o” (for other behaviors and out-of-sight, respectively).

Using JWatcher to Analyze focal animal samples

1. Once you have scored the video clip or clips, analyze the data by tabbing to the Analysis tab.
2. Use the file navigator icon to select the lab.faf—a focal analysis file that specifies the types of analyses we’re going to calculate.
3. Use the file navigator tab to select the data file (e.g., squirrel1.dat) to select the data file to analyze.
4. Specify the Results folder in the Observing Behavior folder as the destination for the results (note: this is automatically created—simply verify that you know where it will be placed).
5. Select: Print results for all behaviors
6. Click the Analyze button at the bottom of the file window to analyze your data.

Viewing the results files

You will need to use Excel (or another spread sheet program) to view the results files. There are two results files, *.cd.res, and *.tr.res. The *.cd.res file has the quantitative results, while the *.tr.res has a file that you can open and graph the “behavioral traces.” For today’s exercise, let’s just open the *.cd.res files.
1. View the *.cd.res file by opening it through Excel. Excel will not automatically recognize JWatcher files; you will need to “tell” Excel to “list all file types” or “show all documents.” The *.cd.res file is a comma delimited text file.

2. Once opened, you should see a list of the behavioral codes and several summary statistics for each behavioral code.
   - N: Occurrence
   - TT: Total Time (in milliseconds)
   - X: Average Time (in milliseconds)
   - SD: Standard Deviation of the average time (in milliseconds)
   - PROP IS: Proportion of Time in Sight

3. Write your results for your focal on the board. Your instructor will combine the results from different focals to create a summary results spreadsheet.

Questions
1. Are the species different? How? Why might this be?
2. How would you more formally test whether there are differences between the species?
3. Data collected to address one question can sometimes be used as preliminary data for other studies. What other hypotheses could possibly be tested that involve measures of vigilance behavior and species differences?
4. We did not talk about errors, or the reliability of observers, but these issues are essential for those scoring behavior. There are two types of reliability that are very important here. Interobserver reliability measures how different observers code the same behavior. Intraobserver reliability measures how the same observer codes the same behavior on multiple occasions. Discuss these types of reliability and suggest ways to quantify them.

Acknowledgements
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Part C: Statistics and Experimental Design

After becoming familiar with how you quantify information about behavior, we now need to look at it more deeply. If you have had a statistics course, you are already familiar with various techniques for data analysis. However, you may not have covered nonparametric statistics, which is especially helpful for small sample sizes and may prove useful to you in analyzing data from your independent research project. If you have had no statistical training, these exercises are designed to be user-friendly, even to the uninitiated.
Exercise 8: An Introduction to Descriptive and Nonparametric Statistics
Elizabeth M. Jakob and Marta J. Hersek

Goals of this lab
1. To understand why statistics are used
2. To become familiar with descriptive statistics
3. To become familiar with nonparametric statistical tests, how to conduct them, and how to choose among them
4. To apply this knowledge to sample research questions

Background
If we are very fortunate, our experiments yield perfect data: all the animals in one treatment group behave one way, and all the animals in another treatment group behave another way. Usually our results are not so clear. In addition, even if we do get results that seem definitive, there is always a possibility that they are a result of chance.

Statistics enable us to objectively evaluate our results. Descriptive statistics are useful for exploring, summarizing, and presenting data. Inferential statistics are used for interpreting data and drawing conclusions about our hypotheses.

Descriptive statistics include the mean (average of all of the observations; see Table 8.1), mode (most frequent data class), and median (middle value in an ordered set of data). The variance, standard deviation, and standard error are measures of deviation from the mean (see Table 8.1). These statistics can be used to explore your data before going on to inferential statistics, when appropriate.

In hypothesis testing, a variety of statistical tests can be used to determine if the data best fit our null hypothesis (a statement of no difference) or an alternative hypothesis. More specifically, we attempt to reject one of these hypotheses. We calculate the test statistic appropriate for our research methods and the design of our study, and calculate the probability that the pattern we see in our data is due to chance alone. This probability is called the $P$ value. By convention, most behavioral ecologists agree that when $P$ is equal to or less than 0.05, we can confidently reject the null hypothesis.

To determine which type of statistical test to use on a given set of data, we must first determine whether or not the data fit a normal (bell-shaped) distribution. If so, we can use parametric statistical tests (see Figure 8.1). If the data are not normal, we must use nonparametric tests. Since many of the data collected in animal behavior studies are not normally distributed, we will focus on nonparametric tests in this lab.

A flow chart to help you decide which tests to use is given in Figure 8.1. Following this is a series of worked examples for a number of nonparametric tests. Begin by acquainting yourself with the flow chart; then skip ahead to the Methods section that follows the worked examples.

Here are some helpful terms:
Ordinal data: numerical data, such as number of seconds, distance, or frequency of a behavior
Categorical data: data that can be put into categories, such as number of animals that moved toward a stimulus, moved away from a stimulus, or stayed in place.

Unpaired data: data points that are independent from each other, such as data generated by testing two separate groups of animals.

Paired data: data points that are naturally paired in some way, most commonly because the same animal was tested more than once. These data points should not be treated as independent from one another.

Number of groups: the number of different test groups being compared.
Figure 8.1. A flow chart to aid in deciding which statistical test is appropriate. Only common tests are included.
After examining the flow chart, look through the following tests. Do not calculate the examples given at this point unless otherwise instructed.

1. Mann-Whitney U test

This test is used to determine the significance of differences between two sets of unpaired data. A ranking system is used.

Example: You are interested in whether the movement rate of the protozoan *Paramecium caudatum* is influenced by whether they are tested under dim or bright light. The null hypothesis is *P. caudatum* has the same rate of movement under both conditions. You measure movement rate by counting the number of squares in a counting chamber a *Paramecium* crosses every 10 seconds.

1. First, order each group from smallest to largest. Next, rank the data of the two groups combined. The lowest score (of both groups) gets a value of 1, the next highest (of both groups) a value of 2, etc. In the case of ties (for example, two values of 12), each value is ranked, the ranks are averaged, and the average rank is assigned to each of the tied scores: \((11+12)/2 = 11.5\). If you’ve done this properly, your last rank will equal \(N\), the total number of samples.

Example: *P. caudatum* movement data (squares crossed per 10 sec.)

<table>
<thead>
<tr>
<th>Dim Light</th>
<th>Rank</th>
<th>Bright Light</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>7</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>9.5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>11.5</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>11.5</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>13</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>16</td>
<td>14</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>17</td>
<td>15</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>9.5</td>
<td></td>
</tr>
</tbody>
</table>

2. Designate the sample size of the larger group as \(N_L\) and that of the smaller as \(N_S\). In our example \(N_L = 8\) and \(N_S = 7\).

3. Sum the ranks \((T)\) of each group.
   \[
   T_S = 7 + 9.5 + 11.5 + 11.5 + 13 + 14 + 15 = 81.5
   \]
   \[
   T_L = 1 + 2 + 3 + 4 + 5 + 7 + 7 + 9.5 = 38.5
   \]

4. Calculate the test statistics, \(U_S\) and \(U_L\).
   \[
   U_S = N_S N_L + \frac{N_S (N_S + 1)}{2} - T_S = 2.5
   \]
   \[
   U_L = N_S N_L - U_S = 53.5
   \]
5. Choose the greater of the two values of $U$. This is the test statistic. Compare it to the critical value in Table 8.2. The test statistic must be higher than the critical value to be significant. In this example, the higher $U$ is 53.5. Look in Table 8.2 under $N_L = 8$ and $N_S = 7$ at the 95% level ($P = 0.05$). The critical value for $P = 0.05$ is 43; since 53.5 > 43, we can reject the null hypothesis with 95% probability that rejection is correct. We conclude that *Paramecium* swim more slowly under bright light.

2. Kruskal-Wallis test

The Kruskal Wallis Test is similar to the Mann-Whitney U test, but here we have more than two groups. Work through the Mann-Whitney U example before attempting this one.

*Example:* You are interested in the antipredator behavior of garter snakes. You wonder how close you, as a simulated predator, can get before the snake crawls away. Because snakes are poikilotherms and can move more quickly when it is warmer, you suspect that this behavior is influenced by temperature. You compare three groups: snakes at 23°C, 25°C, and 27°C. The data are closest approach distance, in meters. The null hypothesis is that snakes tested under these three temperatures do not differ in how close an experimenter approaches before they flee.

1. First order and rank the data, as described for the Mann-Whitney $U$ test. When there are tied scores, each score is given the mean of the ranks for which it is tied. Compute the sum of the ranks for each group, symbolized by $R_i$. $R_1$ is the sum of the ranks of group 1, etc.

<table>
<thead>
<tr>
<th></th>
<th>23°C Rank</th>
<th>25°C Rank</th>
<th>27°C Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1</td>
<td>0.75</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>3.25</td>
<td>6</td>
</tr>
<tr>
<td>1.25</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>4.75</td>
<td>10</td>
</tr>
<tr>
<td>4.25</td>
<td>9</td>
<td>5.25</td>
<td>11</td>
</tr>
</tbody>
</table>

$R_1 = 22$  $R_2 = 37$  $R_3 = 46$

2. Now compute the test statistic, $H$, using the following formula:

$$H = \frac{12}{N(N+1)} \sum \frac{R^2}{n_i} - 3(N+1)$$

In this formula, the $\sum$ is a summation sign, and indicates that you should sum up each $R^2$ value, from $R_1$ to $R_3$. Plugging in the appropriate numbers for $R$, $N$ (the total number of observations), and $n_i$ (the number of observations in each group):

$$H = \frac{12}{14(14+1)} \left[ \frac{(22)^2}{5} + \frac{(37)^2}{5} + \frac{(46)^2}{4} \right] - 3(14+1) = 6.4$$
If you have a large number of ties, use the correction for ties. Compute $H$ as above, then divide by

$$1 - \frac{\sum(t^3 - t)}{N^3 - N}$$

where $t =$ the number of observations in a tied group of scores

$N =$ the total number of all observations

3. Compare your test statistic with Table 8.3. The test statistic must be higher than the critical value to be significant. $H$, at 6.4, is greater than 5.6429, so you may reject the null hypothesis at $P < .05$. The three groups do differ.

**Sign test**

The sign test is used for two-groups when the data are paired. In this test, only the signs of the differences are used. Another nonparametric test, the Wilcoxon matched-pairs signed rank test, is more powerful because it uses both the signs and the magnitude of the differences. We will use the sign test as a general example of how paired data can be treated.

**Example:** You imagine that male mice might benefit from avoiding inbreeding, or mating with close relatives. Because mice depend on odor for a great deal of their information about the world, you decide to present males with soiled litter from the cages of females. You test each male twice: once with litter from his sister, and once with litter from a stranger. The females are sexually receptive, so the soiled litter should be rich in chemical cues. You present the litter in random order so that half the males get their sibling’s litter first, and half get the stranger’s litter first. Since the same males are tested twice, a Mann-Whitney U test is inappropriate. Null hypothesis: The number of sniffs per minute will be the same when males are exposed to the litter of their sisters vs. that of strangers.

<table>
<thead>
<tr>
<th>Male ID Number</th>
<th>Number of Sniffs/Min with Sister’s Litter</th>
<th>Number of Sniffs/Min with Stranger’s Litter</th>
<th>Sign of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>21</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>41</td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>21</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>16</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>18</td>
<td>17</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>11</td>
<td>5</td>
<td>+</td>
</tr>
</tbody>
</table>
1. Subtract one data column from the other to determine the sign of the difference. (It doesn’t matter which you subtract from which, just be consistent.)
2. Note the least frequent sign. In this case, the least frequent sign is negative, and there are two. The test statistic, $x$, therefore equals 2.
3. Determine $N$, the number of pairs which showed a difference. Here we disregard male #11, so $N = 13$.
4. Look at Table 8.4 for $N = 13$ along the left-hand side. Now find $x = 3$. The $P$ value is 0.046 (the initial decimal places are omitted in the table to save space). You can therefore reject your null hypothesis at the 0.05 level.

3. **Chi-square test of independence and chi-square goodness-of-fit test**

Tests using the chi-square statistic are useful when you have nominal data (categories rather than numbers). For example, a category might be “large” vs. “small,” “laid eggs” vs. “did not lay eggs,” etc.

First we will look at the chi-square test of independence. This test helps us determine whether two variables are associated. If two variables are not associated (that is, they are independent), knowing the value of one variable will not help us determine the value of the other variable.

*Example*: When snails sense the presence of a nearby starfish, a predator, via chemicals in the water, they will climb. We can look at three groups of snails: the first group is the control group, with the snails exposed to plain sea water, the second group is exposed to water scented by a sea urchin (an herbivore), and the third group to sea water scented by a predatory starfish. The data collected for each snail is whether it climbed or not. These are categorical data: the snail could do one thing or the other. The categories are mutually exclusive (the snail could not “climb” and “not climb”). If the variables are independent, there will be no relationship between the type of water the snail is exposed to (the first variable) and how it responds (the second variable). Note: if instead of making categories of “climb” and “not climb,” the experimenter had measured the distance each snail moved, the chi-square test would be inappropriate. (Which test should be used for those data?)

1. Make a table of observed frequencies, the data actually collected in the experiment.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Source of Test Water</th>
<th>Row totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Sea urchin</td>
</tr>
<tr>
<td>Climb</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Not climb</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td><strong>Column totals</strong></td>
<td>40</td>
<td>37</td>
</tr>
</tbody>
</table>

Note: The grand total of the rows should equal the grand total of the columns.

2. Calculate and tabulate the expected frequency for each category (for the number of snails observed, the frequency expected in each category if there is no relationship between the variables):
column total x row total
grand total

### Expected frequencies

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Source of Test Water</th>
<th></th>
<th></th>
<th>Row totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Sea urchin</td>
<td>Predator</td>
<td></td>
</tr>
<tr>
<td>Climb</td>
<td>17.2</td>
<td>16</td>
<td>16.8</td>
<td>50</td>
</tr>
<tr>
<td>Not climb</td>
<td>22.8</td>
<td>21</td>
<td>22.2</td>
<td>66</td>
</tr>
<tr>
<td>Column totals</td>
<td>40</td>
<td>37</td>
<td>39</td>
<td>Grand total = 116</td>
</tr>
</tbody>
</table>

3. Calculate the value of chi-square ($\chi^2$):

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

where:
- $O$ = the observed frequency in each cell
- $E$ = the expected frequency in each cell

$$\chi^2 = \frac{(12 - 17.2)^2}{17.2} + \frac{(28 - 22.8)^2}{22.8} + \frac{(14 - 16)^2}{16} + \frac{(23 - 21)^2}{21} + \frac{(24 - 16.8)^2}{16.8} + \frac{(15 - 22.2)^2}{22.2}$$

$$x^2 = 5.97$$

4. Examine the table of critical values for this test (see Table 8.5). The $df$ column corresponds to the degrees of freedom for this test. Degrees of freedom is a number that results from the way the data are organized, and refers to whether the observations are free to vary. For example, if all of 50 observations must fall into two categories, as soon as we know that one category holds 41 data points, then the other category holds nine. For every statistical test, there are established methods for determining degrees of freedom. For the chi-square test, the formula is:

$$df = (#\text{ rows} - 1) (\#\text{ columns} - 1) = (2-1) (3-1) = 2$$

We compare the test statistic to the critical value: if it is bigger, we reject the null hypothesis. The calculated $\chi^2$ is 8.62, which is greater than 5.99. The three groups of snails moved differently.

A second type of chi-square test is called the chi-square goodness-of-fit test. In this case, the experimenter tests to see how the data match expected values that were determined before the test was run. For example, in Mendelian genetics, we can predict the outcome of different crosses; the ratio of the different types of offspring is known in advance. In this case, we compare the observed values from the experiment with the expected values, generated by theory. The calculations are performed in exactly the same way as for the chi-square test of independence.

### 4. The binomial test
This test is useful for categorical data where we have only two categories, and when we are interested in testing whether the data are equally likely to fall into either category.

**Example:** You’ve been using a coin to randomly assign treatments to your experimental animals, but you are beginning to suspect that the coin is not fair, and you decide you’d better test this. The null hypothesis is: the coin is equally likely to come up tails as heads.

1. Flip the coin 11 times. Nine times it comes up heads, and twice it comes up tails.

2. Using Table 8.4, locate the value for N (in this case, 11) along the left side, and the smallest numerical score (x; in this case, 2) along the top. The probability associated with this distribution is 0.033 (i.e., \( P = 0.033 \)). Because \( P < 0.05 \), we can reject our null hypothesis: the coin is not fair.

**Methods**

After you have reviewed the flow chart and glanced through the worked examples (there is no need to rework the examples at this point unless otherwise instructed), attempt the following problems. In each, an experiment is described. Determine which statistical test is most appropriate, and answer all questions posed. Refer back to the worked examples to help you understand how to conduct each test.

1. Elephants make low-frequency sounds, inaudible to humans. Apparently these sounds are used in long-distance communication between individuals. You are interested in the response of bull and female elephants to the sound of a female who is ready to mate. You mount a giant speaker on top of your van and drive around the plains looking for elephants. When you find one, you stop 15 m away, play the sound and watch the elephant’s response. You discover:

   - 9 bull elephants approach the van
   - 2 bull elephants do not approach the van
   - 3 female elephants approach the van
   - 11 female elephants do not approach the van

   Your experiment ends prematurely when one of the bull elephants, apparently enraged by the absence of a female, tips the van over and damages the speaker. You hope that you have enough data to make a claim about males and females.

   a. What is the null hypothesis?
   b. What statistical test should you use?
   c. Calculate your test statistic. Is your result statistically significant?
   d. What conclusion can you draw from this experiment?

2. Male butterflies sometimes court females of other species with similar wing patterns. You are interested in how long males persist in courting the wrong female. You decide to test each male with a dead female, to control for the effect of the female’s behavior. You
use three types of test females: one from the same species as the males, one from a
different species with a similar wing pattern, and one from a different species with a
different wing pattern. Each pair is placed in a cage, and you measure courtship time in
seconds.

Female of same species: 23, 20, 17, 25, 28
Female of different species, similar pattern: 18, 27, 24, 21
Female of different species, different pattern: 22, 21, 23, 20

a. Calculate the mean, variance, and standard deviation for each group.
b. Qualitatively compare the means and standard deviations for each group. (Do they look
very different? Very similar?)
c. Which statistical test would you use to look for differences?
d. Perform the test. What is your test statistic? Can you reject your null hypothesis?
e. Give a biological reason why your test may have come out the way it did.

3. Honeybees returning from foraging convey information to bees in the hive about the
location of food resources. One way they do this is through a waggle dance that other
bees watch. Another way they convey information is by regurgitating some of the food
they have collected to other bees, a process known as trophyllaxis. You are interested in
the speed at which bees find a resource another bee “tells” them about. You decide to
compare bees that have only observed a dance with bees that have observed a dance and
accepted regurgitated food. You mark a lot of bees with bee tags (little numbered discs
that you glue to the back of the thorax). This enables you to watch the same individual
repeatedly. One day you choose a lot of bees that have seen a waggle dance but not
accepted food. You measure (in seconds) how long it takes for them to find the resource.
A week later you go back to the hive, and find the same individuals. This time you watch
until they see a dance and accept food, and again measure how long it takes them to reach
the resource.
Here are your data. The numbers are seconds needed for the bee to reach the resource.

<table>
<thead>
<tr>
<th>Bee #</th>
<th>Watch Only</th>
<th>Watch and Accept Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>87</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>89</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>38</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>160</td>
</tr>
<tr>
<td>7</td>
<td>109</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>78</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>41</td>
</tr>
<tr>
<td>11</td>
<td>67</td>
<td>53</td>
</tr>
<tr>
<td>12</td>
<td>120</td>
<td>98</td>
</tr>
<tr>
<td>13</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>14</td>
<td>89</td>
<td>78</td>
</tr>
</tbody>
</table>

a. What sort of data are these? Which test should you choose?
b. What is the test statistic? The table statistic?
c. You decide that a bee that has both watched and gotten food from another bee finds the resource faster than one that has just watched. What other factor that is a result of your experimental protocol might also explain your results?
Table 8.1 Formulas for descriptive statistics

$Y_i$ is an observation, or data point. The first observation is $Y_1$, the second is $Y_2$, etc.

$N$ is the sample size, or the number of observations.

Mean:

$$\bar{Y} = \frac{\sum Y_i}{N}$$

Variance:

$$s^2 = \frac{\sum (Y_i - \bar{Y})^2}{N - 1}$$

Standard variation:

$$s = \sqrt{s^2}$$

Standard error:

$$\frac{s}{\sqrt{N}}$$

Median: Rank the values from lowest to highest and take the center-most value.

Mode: The most common value.
**Table 8.2** Critical values of U, the Mann-Whitney statistic for $P = 0.05$ and 0.01. (Modified from Table 29, F.J. Rohlf and R.R. Sokal. 1981. *Statistical Tables*, 2nd edition. W.H. Freeman and Company.)

<table>
<thead>
<tr>
<th>$N_L$</th>
<th>$N_S$</th>
<th>$P = 0.05$</th>
<th>$P = 0.01$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>34</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>38</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>43</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>49</td>
<td>55</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>17</td>
<td></td>
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Table 8.4 Table of probabilities associated with values as small as observed values of $x$, for use in sign test and binomial test. Values for total sample size are in the left-hand column, and values for $x$ are across the top. Decimal places are omitted in order to save space.


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Exercise 9: Multiple Approaches to Testing Hypotheses: An Example Using Isopod Moisture Preferences

Matthew H. Persons

Goals
1. To recognize the diversity of experimental designs that can be used to test a single hypothesis
2. To understand the difference between descriptive and inferential statistics
3. To understand the difference between common statistical tests (also see Exercise 8)
4. To practice designing experiments with consideration of the specific inferential statistic used before collecting the data
5. To practice critically evaluating experimental designs

General background
Animal behavior is a quantitative science. This means it is not enough to simply observe behaviors and guess what the animal is thinking, why they are behaving the way that they do, or what it means. Anecdotal accounts of behavior, ad libitum observations, and other preliminary data collection represent a good start, but not the end-product. Animal behavior has few required protocols for answering specific questions. There are almost always many valid methods for testing a single hypothesis. Similarly, there are often different and equally valid ways to statistically analyze behavioral data. In this exercise, we will build on what you have learned in Exercise 8 to design an experiment in order to test a hypothesis about substrate moisture preferences in isopods.

This exercise has two parts. Your instructor may have you do only Part 1, or (over the course of 2–3 labs) complete both Parts 1 and 2. In Part 1, you will draw a statistical test at random, and design an experiment around it. You will use small crustaceans called isopods as your experimental subjects. You will have an opportunity to critique one another’s experimental designs. In Part 2, you will actually perform your experiment with isopods, and analyze the data.

Species background
Terrestrial isopods (Phylum Arthropoda, Subphylum Crustacea, Class Isopoda), are the common woodlice that you can find by turning over stones or logs. They occur in many parts of the world and there are many species. Females of most terrestrial species have brood pouches (marsupia) on the ventral portion of their body that are used to transport larvae for a period of time. Most species are omnivorous and feed on decaying plant, and sometimes, animal matter. Isopods are preyed upon by shrews, a few specialized spider species (e.g., Dysdera spp.) and ants. Isopods have repugnatorial glands that may be used as a predator deterrent. Some, such as many species of Armadillidium, can roll themselves tightly into a ball when disturbed and thus have earned the common names roly-poly or pillbug. Other common species, such as Porcellio scaber, the sowbug, do not roll up. Isopods are one of only a few groups of terrestrial crustaceans and respire through pseudotracheae or “white bodies” that must be kept moist at all times.
Isopods are frequently found to be clustered together in large numbers in particular microhabitats. Why might this be so? You may be able to think of a number of different hypotheses that explain this behavior. For example, isopods might clump together because they are safer from predators when they are in groups, because they are seeking mates, or because they are all seeking out particular microenvironments and thus just happen to aggregate. You can probably think of additional hypotheses. These may not be mutually exclusive.

Each of these hypotheses can generate testable predictions. For example, the hypothesis that isopods are seeking moist environments leads to the prediction that when given a choice between moist and dry soil, isopods will select moist soil. However, remember that they may not be directly attracted to moisture, but instead may be attracted to conspecifics, darkness, cover, particular soil types, fungi, or other cues that are often associated with moisture. Keep this in mind when designing your experiment and interpreting your results.

Ad libitum observations and developing a testable hypothesis

For this laboratory, you will design an experiment to test a specific prediction following from a hypothesis about the aggregation behavior of the terrestrial isopod, Porcellio scaber. You will also use an inferential statistic to help you determine whether or not you should accept or reject your hypothesis.

As in any science, before you formulate a specific hypothesis, you will want to do some ad libitum observations of your subjects. You will have various types of soil available (sand, potting soil, peat moss, and vermiculite), square and plastic containers of various sizes, forceps, paper, scissors, string, a container of water, two stop watches, a spoon for transporting isopods, and, of course, isopods. You will be working in groups of 2–4. The entire class should be divided so that there is a maximum of five different groups. Begin by placing a group of four or five isopods in one or more of the available containers, add some soil, sand, or vermiculite to the container and a small amount of water and watch what they do. A formal testable hypothesis almost always stems from a basic question. Start by developing questions about what the isopods are doing. Where do isopods spend most of their time? Why? Why are they clustered together (or not)? To what environmental cues might they be responding? What can isopods perceive? Can they smell each other? See each other? Discriminate between different types of soil? How do they react when they contact water directly? Of course to move from these general and somewhat vague questions to a formal testable hypothesis will require carefully defining behaviors in an objective way (see Exercise 3, Operational Definitions). If you believe the terrestrial isopod, Porcellio scaber, prefers moist substrates to dry substrates, you may test the following prediction: when given a choice, the isopod, Porcellio scaber, will show a preference for moist soil compared to dry soil. This hypothesis at first glance seems pretty straightforward, but what will you measure to determine “preference”? Time spent on a substrate? Number of isopods that initially moved to a wet substrate? How many times they contacted the wet substrate? With this in mind, your prediction could be rephrased as: the isopod, Porcellio scaber, will spend longer periods of time on a wet substrate compared to a dry one when given the choice. In this example, your measurement of preference, time on a substrate, is the dependent variable or response
variable. The other term to define is moisture. Are you interested in how they respond to saturated or merely damp soil? Isopods may prefer one, and avoid the other, so how much water you add may have a large impact on their response. Moisture level is your independent variable, or what you are manipulating, and is important in determining the various moisture treatments in which you will be recording isopod behavior.

**Statistical background**

When we examine the data we generate, we often begin with *descriptive statistics*. Descriptive statistics provide a summary of the characteristics of the data collected (range, median, mean, variance, standard deviation, and other measures; for further discussion, see Exercise 8). Animal behavior data are often highly variable, as individuals often perform differently from one another (or, indeed, the same individual’s behavior may change from time to time). This natural variation in animal responses may make it difficult to interpret the results of an experiment. *Inferential statistics* (also called *confirmatory*) are used to help you decide whether the pattern you see in your data is a result of chance.

Statisticians use the term “hypothesis” in a slightly different way than we have been discussing in this exercise so far. We’ve been speaking of “hypothesis” as an underlying explanatory idea for phenomena we see, which then generates specific predictions that are tested (see Chapter 1 in your textbook for a more detailed explanation). Statisticians use the word hypothesis in a more restrictive sense, in regard to the specific variables that you have tested. Thus, in statistical language, the null hypothesis is the hypothesis of no difference: the patterns you see in your data are due to chance alone.

Inferential statistics are useful because they provide a probability of being wrong if you choose to reject the null hypothesis of no difference. This probability is usually expressed as a *P value* or probability value. For example, if you analyzed your data using an inferential statistic and generated a *P* value of 0.03, that means you have a 3 percent chance of being wrong in rejecting the null hypothesis that isopods show no preference for substrate moisture level. A *P* value of 0.54 would mean a 54 percent chance of being wrong in rejecting the null hypothesis. In animal behavior, we nearly always use a *P* value of 0.05 as a cutoff point in determining whether or not to reject our null hypothesis. This means that you should have no greater than 5 percent (or one in 20) chance of being wrong. The *P* value that you choose as a cutoff point prior to an experiment is called the *alpha level*.

**Part 1**

You and your group will design an experiment to test whether or not *Porcellio scaber* (or another common species of isopod) prefers moist over dry substrates. One of the mistakes that beginning researchers often make is to design an experiment and collect data, and only then begin to think about which statistical test to use. In some cases, it develops that no test is appropriate. In this exercise, you will be forced to think about your design and the requirement of the statistical test, because you will first select the statistical test and then design the experiment.
Select a statistical test and design an experiment around it. One member of your lab group will randomly draw one of several inferential statistics out of an envelope. Statistical tests are described at the end of this exercise. After reading the description of your chosen inferential statistic, design an experiment that uses that particular statistic. Remember, you should make some ad libitum observations of isopod behavior before deciding what your specific dependent variable will be. Be sure to also test out the various containers and soil types available with live isopods. Sometimes the best experiment conceived doesn’t seem like the best after you have worked with the live animals to be tested. Many animals seem to have a knack for finding the flaws in your design (e.g. can they crawl out of your container? Do their behaviors change dramatically after ten minutes of being in a container? etc.).

Present a proposal of your experiment to the class. After coming up with a design, but before you carry out the experiment, you and your group members will present the methods of your experiment to the entire class. This will serve as a “proposal” of your experiment. Be sure to include materials that you will use, definitions of behaviors that you will measure (see Exercises 3 and 4), and also a mock graph of how you would present your results (including axis labels, units, and a legend, if needed). Your fellow students and instructor will then review your proposal and make some suggestions to improve your design if needed.

Questions concerning each design
Critically evaluating another individual’s experimental design is not always easy, but here are some general guidelines you should consider:

1. Is there a less complex way to answer the same question?
2. What are the possible sources of bias? Could an animal be responding to some stimulus other than that which was intended to be tested?
3. Is the dependent (response) variable an objective measurement (i.e., would someone else score the response the same way that you do)?
4. Were the behaviors to be measured carefully defined?
5. Was acclimation time of the animals allowed for? If so, what rationale did you use for choosing that particular length of time?
6. Is there a way to test the hypothesis such that it more closely approximates natural conditions for the animal?
7. Does the experimental design minimize the harm/discomfort of the subjects?
8. Can you think of another method of testing your hypothesis using the same inferential statistic?

Questions for the class as a whole
After critiquing each design, consider the following:

1. Of these experiments, are some better than others at addressing the hypothesis? If so, why? Does each experiment test the identical hypothesis? Why or why not?
2. What is one advantage and one disadvantage of each experimental design that was presented?
3. What are some reasons that might drive you to select one experimental design over another? Think, for example, of cost, availability of animals, time constraints, etc. Which is most important and why?
4. Did any experimental designs rely upon particular assumptions about the sensory or mental capabilities of isopods? If these assumptions are incorrect, how will that influence the interpretation of the results?

Part 2

Your instructor may then choose to have you carry out your experiment and analyze the data. If so, he or she will ask you to make a list of the materials you need to carry out your experiment.

Statistical tests used in this exercise

As mentioned in Exercise 8, there are two general types of inferential statistics: *parametric* and *nonparametric*. Parametric statistics are used when the distribution of your dependent variable follows a bell-shaped curve (normal or Gaussian). Nonparametric statistics are used when the distribution of your dependent variable does not follow such a curve. In order to determine if your data are normally distributed, you can either use a test available in many statistical packages (your instructor will provide details), or you may graph it. See Figure 9.1 for an example.

Here we briefly review the tests that will be used in this exercise. Complementary material is found in Exercise 8. You will draw from the following categories of tests: two-group unpaired tests, two-group paired tests, tests of more than two groups, and tests with categorical data. Within many of the categories of tests are a parametric and a nonparametric test. If your data are not normally distributed, use a nonparametric test. If your data are normally distributed, you may use either a parametric or a nonparametric test. Parametric tests have more power to detect differences. However, they are also more difficult to calculate, and may require access to a statistical program on a computer. Nonparametric tests are relatively easy to calculate, and directions can be found in Exercise 8. Below are the five choices in the envelope:

1) Simple linear regression
2) Two-sample t-test (parametric) and Mann-Whitney U test (nonparametric)
3) Paired t-test (parametric) and Wilcoxon Matched Sign test (nonparametric)
4) One-way ANOVA (parametric) and Kruskal-Wallis test (nonparametric)
5) Chi-square test and binomial test (both nonparametric)

**Simple linear regression**

*Requirements*: Linear regression is used to examine the relationship between two variables. One variable, called the dependent variable, depends on the value of the other variable, the independent variable. For example, running speed might depend on leg length; however, running speed doesn’t determine an animal’s leg length. Both the dependent and independent variable must be continuous (i.e., real numbers, such as 1, 5,
Examples of continuous variables are measurements of body lengths or the duration that an animal spends engaged in a behavior. (Some variables are categorical, such as whether an animal chose to go toward or away from a substrate. This type of data is analyzed with tests discussed below.)

Data such as these are generally graphed using scatter plots (see an example in Figure 9.2 a).

**Two-sample t-test (parametric) or Mann-Whitney U test (nonparametric)**

**Requirements**: A single dependent variable that must be continuous (e.g., duration of time on a substrate, number of times a subject crosses a line etc.). The independent variable must be categorical (e.g., direction of movement—right or left, sex, etc.) and consist of only two treatments. Each individual animal is tested only once.

Data like these are often illustrated with bar graphs or box-and-whisker plots (see Figure 9.2 b and c).

**Paired t-test (parametric) or Wilcoxon matched sign test (nonparametric)**

**Requirements**: The dependent variable must be continuous (e.g., duration, number of times an animal crosses a line, etc.). Independent variable must be categorical and consist of only two categories (e.g., experienced-inexperienced, male-female etc). Individuals from each of the two categories must be “matched” in some way or an individual test subject will be tested under two different treatments. A paired t-test is very similar to a two-sample t-test except that the two groups to be tested are correlated or related in some way. For behavioral studies, it is usually used when the same individual is subjected to two different treatments. For example, you may test an animal’s foraging speed when it is hungry vs. full, or the length of time it takes an animal to complete a task before vs. after training. Because individual animals often consistently differ in their behavior (e.g., some rats are always slower at learning than are others), a paired t-test allows us to better isolate the effect of treatment. Paired t-tests are also used when pairs of subjects in two different groups are “matched” on the basis of some variable like age or length of training to ensure that the pairs of subjects in each group are the “same” prior to an experiment. For example, if you wanted to test for differences in the time spent engaged in play behavior among male and female cats, you may choose to compare pairs of a male and female that are in the same age category since play behavior may vary significantly with age. A paired t-test requires that both groups have the same number of measurements taken or responses since either the same individual is tested twice, or the pairs are matched in some way.

**One-way ANOVA (analysis of variance) (parametric)**

**Kruskal-Wallis test (nonparametric)**

**Requirements**: The dependent variable must be continuous and non-categorical (e.g., duration on substrate). The independent variable must be categorical and consist of more than two categories. Each individual is tested only once.

Notice that the requirements are identical to the two-sample t-test except that the independent variable has more than two treatments. For example, if you wanted to test if younger rats were faster than older rats, you may choose to divide rats up into four
different age categories instead of just two: 6 months old, 1 year old, 2 years old, and 3 years old. As another example, you may be interested in testing how long bees spend on artificial flowers of four different colors, red, yellow, green, and blue.

**Chi-squared test or binomial test (nonparametric)**

*Requirements:* The dependent variable must be categorical and follow a binomial distribution (presence/absence, yes/no, 0-1, etc.). The independent variable must be categorical. The independent variable may consist of two or more categories.

The chi-square test and binomial test are both widely used and easy to compute, as described in Exercise 8. To review, for many experiments, the dependent variable of interest is categorical. As an example, let’s suppose we were interested in whether or not isopods have an initial turning bias toward the left or the right. If you placed individual isopods in a T-maze and recorded whether an individual turned right or left, you would place all individuals in one of the two directional categories. In this case the dependent variable is simply the number of isopods that turned one direction or the other. Since this data is not continuous, it must be nonparametric. If the expected distribution of isopods that turn right versus left is 50/50 and you only have two such categories, then a binomial test is appropriate. If you wanted to see if a coin was fair, you would use a binomial test to determine this. Many types of data have dependent response variables that are binomial and fall into only two categories (yes/no, male/female, left/right, etc.). If you have more than two categories (e.g. blue, green, yellow) for your independent variable, or if the expected distribution is other than 50/50, then a chi-square test would be appropriate. For example, if isopods had three choices in a maze and you wanted to test for a choice bias among the three (right, left, or straight) the expected distribution would be that 33 percent of your isopods sampled would choose each.
A. A histogram with a normal distribution laid over it. These data are very close to being normally distributed, and using a parametric test to analyze them would be fine.

B. The data illustrated here are not normally distributed, and should be analyzed with a nonparametric test. (Note that transforming the data or using a distribution-free bootstrap test is also possible, but are beyond the scope of this manual.)

Figure 9.1 Distributions of data.
Figure 9.2 Three different methods of presenting quantitative data.

A. Scatter plot to show relationship between two variables.

B. Bar graph to show differences between two graphs. The error bars indicate the standard error of the mean.

C. A box-and-whiskers plot. The ends of the box are the 25th and 75th quartiles (25% of the data points lie below the bottom line and 25% lie above the top line). The line in the middle of the box is the median. The “whiskers” extend to the outermost data points.
Exercise 10: Designing Testable Hypotheses
Marta J. Hersek and Elizabeth M. Jakob

Goals
1. To practice designing experiments using organisms and tools at hand
2. To collect and analyze data as a team
3. To put together a presentation of your data

Background
There are a variety of methods available to us to study animal behavior. Which method we use depends upon such facts as which organisms we are studying, the behavior of interest, the number of organisms available, the situation in which the organisms live (e.g., in the laboratory or in nature, etc.). We’re often interested in how two or more groups of organisms differ, or in how a group of animals change their behavior under particular circumstances. In nature we may use observational techniques alone to study the animals’ behavior, but in the laboratory we usually are able to perform experiments. The latter method is the focus of this lab.

When we set up an experiment, we may be interested in how a particular variable, such as a certain procedure, affects the animals. In this case, we are careful to have an experimental group, which undergoes the procedure, and a control group, which does not but is otherwise treated exactly the same as the experimental group. Similarly, we may want to study animals in two different sets of environmental conditions. Finally, we may be interested in how organisms behave before and after a particular occurrence. In this case, the organisms act as their own control group. The details of the experimental design will determine which statistical test we will use to analyze the data.

Once we have determined what our study entails, we can formally state our hypotheses. The null hypothesis is a statement of no difference. That is, a null hypotheses states that the two (or more) groups which we are studying do not differ with respect to the behavior of interest. An alternative hypotheses says that one group will differ from another in a particular way. Our goal is to design our experiment so that we will be able to reject the null hypothesis, and provide support for an alternative.

In this lab, you will work in teams to design an experiment, collect the data, analyze it, and report your findings. You have a variety of organisms and equipment to work with. Ideally, animal behaviorists study a particular species for a substantial period of time before deciding on which hypotheses to test. However, in this laboratory we will emphasize rapid generation of testable hypotheses. Below is some background information that will help you formulate hypotheses that are likely to be relevant to the animals provided.
Species that may be available

1. The large milkweed bug (Oncopeltus fasciatus)

Milkweed bugs are sucking insects in the order Hemiptera. They eat seeds by inserting their proboscis into the seed coat. They are hemimetabolous insects: nymphs and adults resemble one another, except that adults have wings.

In nature, milkweed bugs eat—surprise—the seeds of the milkweed, a common roadside plant. Milkweed contains cardiacglycosides, and milkweed bugs and other insects that consume it (such as monarch butterflies) are poisonous to many potential predators. Many poisonous insects have aposematic, or warning coloration. Typical warning colorations are red and black or yellow and black. Apparently these combinations of colors are highly visible and easy for many birds and other predators to learn. Many aposematic insects clump together in groups, which may serve to enhance visibility.

Female milkweed bugs in nature lay their eggs in crevices between milkweed pods. Often females lay 30 eggs a day. In the lab, milkweed bugs readily oviposit in cotton.

Milkweed bug adults are migratory, and they fly hundreds of miles southward to escape bad weather in the fall. Nymphs, of course, do not migrate, since they do not have wings.

Our milkweed bugs have been fed unseasoned sunflower seeds. Initially, survivorship on a novel food source will be poor, but over several generations a line of milkweed bugs adapted to the new food can be developed. You may have access to both nymphs and adults.

Handling milkweed bugs:

Milkweed bugs do not bite. Pick them up carefully, with your fingers or with soft forceps. Hold them firmly without squeezing. Petri dishes with lids can be used for temporary storage and transport of bugs during the laboratory period.

2. The confused flour beetle (Tribolium confusum)

Flour beetles are holometabolous insects: the larvae and adults differ greatly, so they undergo complete metamorphosis. Flour beetles are considered one of the most serious insect pests of cereal foods, but closely related species are found in rotten logs and in leaf litter. They are commonly used for experiments on population growth and for food of frogs and other lab animals. Tribolium are cannibalistic: adults eat eggs, and larvae eat eggs, pupae, and other larvae.

Handling flour beetles:

A hand-held sifter is provided. Scoop up some flour and shake over the box to get the flour out. You will be left with larvae and adults. Beetles can scurry away quite rapidly, so be careful not to let them escape. A petri dish can be used for transportation.

3. Field crickets (Gryllus sp.)

Crickets are hemimetabolous insects that show a number of interesting behaviors (see Exercise 4). In nature, crickets are territorial and will defend burrows. In enclosed
spaces, such as aquaria, they set up linear dominance hierarchies (A is dominant over B and C, B is dominant over C, C is subordinate to both A and B). Adult males “sing” by rubbing a file and rasper on their wings together to attract mates. They also have a soft courtship song which they employ when females get very close. Juvenile crickets can be distinguished from adults because they do not have wings. Adult males and females are easily distinguished by the presence of a long ovipositor extending from the rear of the body on female.

Handling crickets:
Crickets do not bite. If they are in a large container, capturing individuals with a plastic cup works well. Pick them up carefully with either your fingers or soft forceps, being careful not to squeeze them. Their jumping legs can be easily broken off, so take care.

4. **WOW bugs** (*Melittobia digitata*)

WOW bugs are small parasitic wasps. In nature they parasitize a variety of hosts, with mud dauber wasp pupae being a favorite. The males of this species are blind, and they result from unfertilized eggs (as is common in Hymenoptera). They vigorously pursue females with an elaborate courtship, and just as vigorously fight other males. Fertilized females lay eggs that result in 95 percent female offspring; unmated females lay a few eggs on a host, then mate with the resulting males before laying a large number of eggs on the same host pupa. These wasps often hop along the substrate, rather than fly.

Handling WOW bugs:
WOW bugs don’t bite, and can be tipped from one container to another without difficulty.

5. **Wolf spiders** (various species)

Wolf spiders are member of the family Lycosidae, and are among the spiders that do not build webs. Instead these animals actively hunt and pursue prey. They eat a variety of prey types. They have very good eyesight, and communicate through vision, pheromones, and vibratory behaviors.

Handling spiders:
Spiders can bite if handed roughly. They also move very quickly. It is best not to pick them up with fingers or forceps, but rather either scoop them up into a vial, or use a small paintbrush to herd them.

6. **Brine shrimp** (*Artemia sp.*)

Also known as “sea monkeys,” these small crustaceans are known for their ability to tolerate a wide range of environmental salinity. They are found in shallow bodies of water throughout the world, and are an important food source for many other organisms. They can also tolerate desiccation by enclosing themselves in a capsule. The right conditions will then cause the organism to emerge from this protective structure. These aquatic
organisms are easily visible to the naked eye, and they show preferences to particular conditions by swimming to appropriate areas of their container.

Handling brine shrimp:
Carefully pour the water with the shrimp in it from one container to another. Be careful not to leave individuals stranded on the sides of a container.

**Specialized equipment**
Most of the materials that you will be provided will have obvious uses, and we will leave it to you to figure out how best to deploy them. One not-so-obvious piece of equipment may be mazes that can be used to test a variety of questions about how animals move. The mazes are pictured below. They are made of wooden dowels, and animals carefully placed on one end can make their way to the other side. These work best with milkweed bugs. There are two forms of mazes:

**T mazes.** T mazes are typically used to see whether animals avoid or are attracted by a particular stimulus, such as a bright light. The animal is place at the bottom of the T and makes its way to the choice point.

**L-T mazes.** Animals need to continue in a straight line if they are to move rapidly through their environment. Many insects can remember the direction they have been walking. If they are forced to turn “off course” in a particular direction, they can often remember to turn back the other way when confronted with a choice. This is the function of the L-T maze: first a turn is forced, then the animal has a choice. Some species quickly forget a course they have set. If they are forced to travel a longer distance after the forced turn, they “reset” to a new course. The large and small L-T mazes can be used to test this question. Other species can remember for a longer period.

![T maze and L-T maze](image)

**Methods**

**First lab period**
In the first lab period your group should design an experiment. For example, if you wish to test that milkweed bugs remember the direction in which they are travelling for only a short distance, what will you predict about their performance on the small and large L-T mazes? As you design your experiment, be sure to think about establishing adequate controls. For example, bugs might also respond to a light source. How can you design your experiment to control for this problem? Decide in advance which statistical test you will use to analyze the data, and formally state the null and alternative hypotheses.
that you will test (see Exercise 9 for a more thorough discussion of the different ways the word “hypothesis” is used). Do the requirements of the statistical tests influence how you will design your experiment?

Before you finalize your decision, run some practice tests. Make sure that the animals and equipment you have chosen are appropriate. It will be very unusual if your initial plan works exactly as you expected, and it is likely that you will have to modify your design. When you are satisfied with your methods, begin collecting data.

Your instructor may wish you to check with him or her, or present your plan to your classmates.

**Second lab period**

In the second lab period, you should continue to collect data, if necessary. How much data should you collect? Often behaviors are not highly predictable. You may need a reasonably high sample size to see statistical significance in your results. One of the objects of the exercise is to get a feel for how many trials you might need to demonstrate a phenomenon. If you are studying a predictable behavior, you might be able to reject your null hypothesis with a fairly small sample size. If there is a lot of variation in the behavior, you need a larger sample size to document the existence of a pattern. The larger your sample size, the more certain you are that you are accepting or rejecting your null hypothesis with good reason.

Analyze your data. Can you reject your null hypothesis? Calculate descriptive statistics, so that you will be able to present your data effectively.

By the end of lab your group should be prepared to present a summary of what you did. Include the design of your experiment, and the results of your statistical test. You may use overheads to present your data, or draw a graph on the board. The presentation should be short and sweet (5 minutes). Speculate (without going too far afield) about what your results mean.

**Report instructions**

At home, each person should write two sections of a scientific report for your experiments: the Methods and Materials section, and a Results section.
Part D: Interpretation and Presentation

A scientific project is not complete until the results are made public. Scientific writing and other forms of scientific presentation differ in many respects from other sorts of writing, so these differences need to be explicitly addressed in your science courses.

In this section, we present advice for presenting your data in four different formats: a paper, scientific talk, a poster session, and on a web page. Included are some exercises for refining your skills at presentation.
Exercise 11: Scientific Writing

Elizabeth M. Jakob

Goal
To become familiar (or reacquaint yourself with) the contents of different sections of a scientific paper

Background
There exist many fine books on the art of writing a scientific paper, and even more on perfecting your writing style. Here I will review briefly the different parts of a scientific paper. For more details (and other perspectives on what’s important), please consult the material cited at the end of this Exercise.

Scientific papers are not literary works. Instead, they are meant to transmit information effectively and concisely. There is a very explicit format that all papers must follow, with small variations in style among journals. There’s no option, for example, for surprise endings in scientific papers—the answer is always given in the first section! Some students become frustrated with the strictness of these rules, but it can be quite satisfying to write clearly within the confines of the standard format.

Papers are broken down into the following sections. Every section, except the title, should be labeled. Generally the section name is centered and underlined (or bold-face) over the text.

Title. The title should give the reader a concise, informative description of the content and scope of the paper.

Abstract. This is a concise summary of the major findings of the study. It is generally no longer than 9–10 sentences, or half a page. It should summarize every subsequent section of the paper. (A classic beginner’s error is to summarize everything except the Discussion section.) It should state the purposes of the study, briefly summarize the methods, major results, and major conclusions of the study. The abstract should stand alone: do not refer to any figures or tables, or cite any references.

Generally scientists write the abstract last, because you need to know exactly what is in the paper before you can summarize it.

Introduction. This section gives the rationale for the experiment. It answers the question “Why should I care?” It usually includes background information, including the work of others, and a description of your objectives.

Give both the scientific (Latin) name of your species, and the common name. The scientific name is always underlined or italicized, and the genus name is capitalized while the species name is not.

Cite only references pertinent to your study. Direct quotes are rarely used in scientific writing; instead state the findings of others in your own words. Footnotes are not used in scientific papers. Instead cite the author by last name, and the year that the source was published.
Tinbergen (1960) was first to study the function of the red spot on the bill of herring gulls.

Herring gull chicks peck vigorously at the red spots on their parents’ beaks (Tinbergen 1960). (Some journals put a comma in between the author and date, while others do not. Your instructor may have a preference. If not, be consistent throughout your paper.)

When two people co-author a paper, both are cited.

Hamilton and Zuk (1982) proposed that parasites play an important role in the evolution of mate choice.

When more than two people coauthor a paper, cite only the first, and follow with et al.

Krebs et al. (1977) used a conveyer belt reminiscent of those that carry airport luggage to test foraging behavior of birds.

The full reference for each work must be given in the literature cited section at the end of the paper. Cite work from the primary literature: that is, work that is published by the same people who did it. Do not cite encyclopedias, textbooks, etc.

When organizing your introduction, begin broadly and then narrow your focus. For example, a project on mice’s use of olfaction to locate food might be organized as follows:

Many animals face the problem of locating food that may be hidden from view.

Mammals rely heavily on odor cues to find food…

Little is known about the ability of spiders to use odor cues in locating food…

The aim of this study was…

Each of these sentences would be a good topic sentence of a different paragraph in the introduction. In sum, an introduction should convey your overall purpose in conducting the experiment as well as your specific objectives. Cite references to place your study in the framework of the literature.

**Materials and Methods.** This section is a very concise summary of the subjects, equipment, and procedures used. This section should contain enough information so that someone else could repeat your experiment. It is *not* a list, but a narrative description. Be sure to include, where relevant, information on the number of study animals, their sex and age, equipment, methods and duration of observation (scan sampling? focal sampling?),
which statistical test you used, etc. It is often helpful to break this section into subsections, such as Experimental Subjects, Apparatus, Procedure, and Statistical Analysis. If you are following the methods of another paper or a lab manual, cite the source. A common mistake is to let results creep into this section.

**Results.** This section includes presentations of your data and the results of statistical analysis of your data.

First state the overall trend of the data. For example, if your project is on the interactions of goldfinches at a feeder at different times of day and in different weather conditions you might begin by stating “Goldfinches engaged in more aggressive interactions in the morning than in the afternoon.”

Address each statistical test separately, often in separate paragraphs. For each analysis say whether your results are statistically significant, and in parentheses give the statistical test used, the value of the test statistic, and the probability level for that computed value. For example, “Male mice visited non-pregnant females significantly more often than pregnant females (chi-square test of independence, \( \chi^2 = 4.69; P < 0.05 \)).”

Do not present your raw data. Instead, present data in an easy to read form. You will probably use a figure or a table to present your results. Each table is referred to by a number (Table 1, Table 2, etc.) Each should have a concise legend at the top. Graphs and diagrams are both called figures and are numbered consecutively (Fig. 1, Fig. 2, etc.) They have legends at the bottom. Clearly label your figure axes.

You must refer to every table and figure at least once in the text. Often this can be done parenthetically: “Male mice visited non-pregnant females significantly more often than pregnant females \( \chi^2 = 4.69; P < 0.05; \) Fig. 2.” Do not use the word “significant” unless it can be supported by statistical evidence.

A common mistake is to let discussion creep into this section.

**Discussion.** Here you are to give a reader the “take home” message of the study. Begin by briefly summarizing the major findings of your study. Then discuss each finding one at a time (usually in separate paragraphs).

Interpret your results in light of the animal’s biology. Your discussion section should parallel your introduction: if you discussed the role of reproductive biology of the mouse at the beginning of your study, come back to it again here. The paper should come full circle. Cite references throughout your discussion to support your points and for comparison to your data (thus, in general, most references will be cited in either the Introduction or Discussion, with the exception of references about methodology). Do not make statements that cannot be supported by the data. Discuss possible errors in the experiment, but don’t make the common mistake that beginning writers often do, of focusing completely on potential errors in your work.

**Literature cited.** A quick browse through different journals that publish animal behavior articles will demonstrate that this section is the most variable in style, although generally the same components are always present (author, year of publication, article title, journal title, journal volume, page numbers). Your instructor may give you a particular model to follow, and it’s important to pay attention to the details of the format. For example, what
is the style of indentation? Is the journal name italicized or not? Is there a colon, a comma, or nothing between the journal volume and the page numbers? Many authors submitting their manuscripts for publication have discovered the hard way that inattention to these details can slow down a paper’s acceptance, as editors are not interested in fixing careless mistakes. If your instructor does not give you a particular model to follow, you should at least be internally consistent and use the same format throughout. Here are some examples of a standard format:

Journal article: The title of the article is not capitalized or underlined. The title of the journal is capitalized. Next comes the volume number, then the page number.


Books: The title of the book is capitalized and underlined or italicized. Next comes the publisher and the city of publication.


List the citations alphabetically by the last name of the first author.

**General hints.** Remember, just because you are a good writer in your literature classes doesn’t mean your skills will translate to science writing without work. Scientific writing can be challenging: the ideas are complex and the level of detail you need to include can be daunting. It can be hard to communicate clearly to the reader. It is helpful to keep in mind some stylistic guidelines suggested by Gopen and Swan (1990). Readers interpret your writing and deduce meaning in accord with expectations that they have about the structure of prose. For example, readers expect that a subject is followed by a verb. If you make the common mistake of constructing a complex sentence where the verb is separated from the subject by a great many other words, the reader may lose focus. Readers also tend to place stress at the end of a sentence. This is generally where new information that you wish to emphasize should go. Old information that serves a transitional role should generally go at the beginning of a sentence. By keeping a reader’s natural tendencies in mind, you can convey complex information more effectively.

If you have had experience writing scientific papers in other courses, particularly psychology courses, be alert to differences in style in this course. Somewhat annoyingly, even though the general format of scientific papers in biology is standardized, the details vary across journals even within a discipline for scientific papers. One of the first things an author of a scientific paper does is to locate the instructions to authors for the particular journal that is targeted for submission, and make sure that the formatting style is followed. It is the author’s responsibility to follow the instructions for a particular journal. Similarly, it is your responsibility to follow the instructions for papers in this course, even if they differ from those in other courses.
Outline your paper. Use topic sentences for every paragraph. Someone else should be able to go back and underline each topic sentence. Check your paper using the list provided at the end of this Exercise.

**A short exercise in scientific writing**

Your instructor will provide you with an envelope that contains two items: index cards with the major sections of a scientific paper, and clips of sentences from actual scientific papers. Your task is to work with a partner to match the sentence to the section. Begin by spreading out the index cards, and then work through each sentence one by one, placing them underneath the appropriate card.

1. Were sentences fairly easy to categorize?
2. If there were some that were unclear, which sections were you trying to decide between (e.g., Introduction vs. Methods, Introduction vs. Discussion)?

Next, get from your instructor a copy of the complete article, and check your work.

3. If you made mistakes, where were they? Why do you think this is so?

**Other sources**

[The following focus on scientific writing.]


[The following address general issues of style and mechanics, and are great references to have.]


Checklist for Paper and Poster Writing

You should be able to answer “yes” to the following questions.

**Title**
_____ Is it concise and informative?

**Abstract**
_____ Is it the proper length?
_____ Is each section of paper summarized?
_____ Does it omit references to figures, tables, or other work?

**Introduction**
_____ Organization: Does it begin with the “big picture,” then narrow?
_____ Is it clear why you chose to do this experiment?
_____ Is the scientific name and the common name of your species given?
_____ Are references to other literature given?
_____ Are references cited correctly?
_____ Are the objectives of the study clearly evident?

**Methods and Materials**
_____ Is it written in narrative form?
_____ Does it include enough information so others can repeat the experiment?
_____ Is it clearly organized?
_____ Does it refer to the Methods of earlier work when appropriate?
_____ Are Results omitted from this section?

**Does it include information about**
_____ The study animals?
_____ The apparatus (if appropriate)?
_____ Experimental design?
_____ Methods of data collection?
_____ Statistical tests chosen?

**Results**
_____ Are the main results clearly summarized in a paragraph?
_____ Are raw data omitted from your Results?
_____ Are graphs and figures clearly labeled and properly presented?
_____ Is every graph and figure mentioned in the text of the Results?
_____ Are the results of statistical tests presented parenthetically?
_____ Have you avoided discussing the results?

**Discussion**
_____ Are the major findings clearly summarized?
_____ Are the results properly interpreted?
____ Are questions raised in the Introduction clearly answered?
____ Are references cited to support your points?
____ Are possible errors discussed?

**Literature Cited**
____ Is everything listed cited in the paper, and everything in the paper listed here?
____ Are the citations in proper format?

**Overall**
____ Does every paragraph have a topic sentence?
____ Is every sentence written as concisely as possible?
____ Is every section clearly organized?
____ Has the paper been carefully checked for spelling and grammatical errors?

This checklist is based on one that can be found in the student’s instructions for Introductory Biology, Cornell University, circa 1979.
Exercise 12: How to Give a Talk
Elizabeth M. Jakob and Adam H. Porter

Goals
1. To learn which information should be included in a scientific talk
2. To consider matters of presentation style
3. To become familiar with at least one type of visual aid

Background
The purpose of a scientific talk is to convincingly explain a scientific finding to the audience, using an argument constructed with logic and evidence. It’s not “just a lab report” where you simply describe what you did and what resulted; your goal is also to relate your findings to the scientific literature—that is, explanations that other scientists have discussed for the phenomenon you are studying. Here we present some advice for preparing an effective talk.

Questions to ask your instructor before you prepare your talk
• Who will your audience be? If it is your classmates that have worked on different aspects of the same project as you have, you will include a different level of detail than you will if your instructor has invited people from outside your course to see your talk. If your audience includes people with less exposure to your topic, you probably ought to pay special attention to the scientific vocabulary you use: pare back to only the jargon that is absolutely necessary, and include brief definitions even of the simple terms.
• How long will your presentation be? It is vitally important not to go over your allotted length. Both in the classroom and in scientific meetings, you may rightly be interrupted and stopped once you have reached your time limit, even if you aren’t done talking.
• Which visual aids are available to you? How polished is your presentation meant to be? (Are overheads written in marker OK, or are you to have elegant PowerPoint slides?)

The structure of the talk
Start with the “big picture.” Unless you are speaking to a room full of specialists, most of your audience won’t be clued in to the nuances of why your topic is interesting. So, you need to explain why they should care. The first minute or two of a talk are arguably the most important for holding their attention. Follow an “hourglass” structure as a general rule: start with the big picture, narrow the focus, and leave time at the end to return to the big picture. Thus, the old standby advice for any public speaking is still good: tell them what you are going to say, say it, and then tell them what you said.

In the beginning of your talk, you should put your experiment in context. Why is your hypothesis of scientific interest? How does your explanation of your results provide new insight, or help confirm a previously proposed explanation? If you are testing a particular hypothesis in the literature, you should cite the person that proposed it. Unlike
a written paper or poster, you will not have a literature cited section in your talk: your job is to clearly lay out the ideas behind your research.

Next you will present your methods and results. Hints for presentation are below. In these sections, clarity and organization are exceptionally key.

After you present your results, make your conclusion. Your conclusion should relate back to your introduction. Never make a conclusion that is stronger than the evidence you present, no matter how elegant or intuitively satisfying you think it is. You may suggest an explanation that goes beyond your data, but acknowledge that you are doing so. You don’t have to come up with “the explanation” of a phenomenon; it is a solid piece of science to simply eliminate one possible explanation from a list of them. By the same token, it is a rare experiment that gives exactly the desired results, and most experiments yield surprises of one sort of another. These surprises aren’t necessarily evidence that your experiment “failed.” In fact, trying to explain such surprises often leads scientists to important new insights. For your experiment, these surprises can point towards additional factors that might be involved and therefore, more experiments that you can suggest should be done (e.g., perhaps the males seemed to have behaved differently than the females in your experiment, and you might be able to think of a few reasons why this could be true). So, never apologize for your research (besides, no one wants to hear that you are wasting their time!). Instead, go ahead and point out the next steps that might be needed to sort among any remaining explanations for your results.

It is traditional to end by thanking people who helped you significantly with the work (but don’t go overboard—a few names on a slide are fine), and then asking for questions from the audience.

**Visual aids**

Most people think visually, so visual aids well-placed in your talk will help them quickly understand things that could take several minutes to explain otherwise. These visual aids might include images (photographs or simple line drawings, even animations or movies), but also include more subtle things like changes in font size or color that you can use to emphasize written content. Fine talks can be given with overheads written in pen, or with presentation software with fancy animations—but poor talks can also be given in each medium! You will likely be using either computer-based presentation software, such as Microsoft PowerPoint, or overheads for your talk. For convenience’s sake, we’ll refer to these as “slides.”

Your visual aids should reflect the organization of your talk. If you are doing a computer-based presentation, it is natural to have all components of your talk reflected in your slides (the introduction, methods, etc.). If you are using overheads, you may want to talk through the introduction without using overheads, but rely on them for illustrating the design of your experiment, the results, and the like. Whatever format you choose, never be surprised by your own slides (“Oh, wait. I forgot I put that stuff in!” “Why did I put that slide here?”).

Many people like to use explicit organization or question slides. For example, if you are discussing three possible hypotheses, list them on a slide at the beginning of your talk. An effective technique, particularly in long talks, is to repeatedly return to a copy of this original list, with a marker at the topic currently under discussion.
Experimental methods often benefit from illustration. For a complicated apparatus, present either a photograph or a diagram; a diagram followed by a photograph works especially well (it is very straightforward to insert a digital photo into a PowerPoint talk using the Insert Image from File command). For a complex experimental design, a figure is invaluable, such as a flow chart illustrating what you did. Your audience will be trying to follow your scientific logic throughout the talk, and to understand your results later, they will need to clearly understand your methods at this stage of the talk. Good diagrams will be well worth your effort.

Numerical data must be easy to read. Slides should contain only the numbers you will actually discuss. Six or eight lines of data are about as much as anyone can digest; if you need more, then you should probably think of a way to make a graph of the data instead. Never gesture at a slide full of numbers and say “Ignore all this; here’s the number I want you to get.” If you do this, you should have remade the slide.

When possible, present your data visually, in graphs rather than in numbers. On graphs, label your axes in a big enough font that people can read them, and even then, tell the audience what the axes are.

Use a large font. Many people have vision problems of one sort or another that makes it difficult to see things from the back of a medium sized room, and it is hard to make a font too big. No one will ever complain that your slides are too easy to read! For an average laboratory room, use a minimum font size of 18 points, and don’t be afraid to go quite a bit bigger. As a rule of thumb when preparing your slides, your font should be clearly legible on the computer screen from 8 feet away. Keep your range of font sizes uniform from slide to slide. Also, in our experience, it is wise to use the common fonts (Helvetica, Geneva, Times New Roman, Symbol, etc.) and stay away from the fancier ones. Partly this is because some people in your audience will find the fancier ones harder to read. But, the main reason is that if you give your presentation on a different computer than the one you used to create it, the new computer might not be able to present that font correctly.

Be careful with color. Too little color is boring, but too much color creates chaos that you don’t need. It becomes difficult to read and will draw your audience’s attention away from the point you are actually trying to make. Especially distracting are the nifty shapes and backgrounds that are provided as templates in presentation software—your audience may daydream and trace the pretty shapes with their eyes instead of attending to what you are trying to say! We prefer very simple backgrounds, consistent among slides, and we try to avoid using more than about three different colors on a single slide. Even so, color can be a powerful tool for you, and can help you explain some concepts that are otherwise very difficult to handle succinctly. A new color can help you smoothly call attention to part of a slide (“First look at the line in yellow on the graph …”) when you need to help your audience break down a complicated idea into bite-sized pieces.

You should also pay attention to which colors you use. Colors with the same “saturation value” (basically, colors that filter or reflect the same amount of light, and so do not contrast much) are often hard to distinguish. For example, green lettering on a blue background often doesn’t show up in a presentation, even when it shows up well on your computer screen. One trick you can use is to lighten the color of the lettering to a very pale color; the audience’s eye will adjust so that even a very pale red will look like
saturated red on a black background, whereas a saturated red will seem to flicker and be hard to read. And, remember that about 3 percent of the human population is red-green color blind, and won’t be able to distinguish some of the colors you use. Try to avoid using red and green in the same slide, especially if you are comparing things with different colors.

Cool graphical tricks (animated text marching across the screen, spinning three-dimensional graphs, whatever) generally ruin an otherwise perfectly good scientific talk. Your talk will be most impressive if you use the simplest visual aids that you need to place the audience’s attention where you want it and illustrate your points clearly.

Style

Don’t get too far removed from your natural way of talking. Obviously you want to reduce slang and use precise terminology, but you want to make a connection with the audience that can be difficult to achieve if you are very formal.

Don’t read. Speaking style is different than writing style. A reading audience can follow a complex sentence structure that would give a listening audience problems. Carry notes if you want them for confidence, but try not to look at them much. Instead, practice the talk.

We prefer talks that are not memorized word for word. Even so, you will find that some parts of your talk require precision of speech, and you may want to memorize those parts. Other parts you may approach slightly differently each time. Try memorizing the first few paragraphs of your talk to get you through your opening jitters. After that, the visual aids should help to carry you through the organization, and remind you of where you are if you get lost. Practice your talk until you are comfortable with it. Some find it is possible to over-practice.

Try to convey your enthusiasm for the problem, especially in those first two minutes of the introduction. It will help retain the audience’s interest, and help you avoid being self-conscious. If you hated your project, at least take pity on your audience!

Joke if it is natural. Be sure not to offend anyone.

Be aware that many people dramatically speed up their rate of talking under stress! If you know you do this, then practice pausing at specific, pre-determined spots in your talk. And, run through your talk out loud several times before you give it in public. This will increase your familiarity with the content and flow of the talk, and decrease your stress. And if it helps, remember that the talk isn’t about you, it’s about the science.

Make sure data slides are up long enough for people to read for themselves. If you have something particularly important, say “This is particularly important, so I want to spend some time explaining this.” Do not be afraid to tell people when they should pay close attention. They will appreciate it.

Never put a slide up before you are ready to talk about it. The audience will be reading the slide, not listening to you. Use blanks, or simple filler slides with pictures of your organism or study site (make sure these filler slides are not confusing, though).

Interact with your slides. Point to the area you are talking about. Photographs that may be very clear to you are often not so clear to others; point out the animal if it’s not obvious. If you are given a laser pointer, use it sparingly and hold it steady or move it slowly when it is on. Your audience’s eyes are rarely following your thoughts as you talk,
and slow steady movement helps them do so, but rapid movement attracts the eye so much that the point you are trying to make will be lost. So, don’t wave the pointer around to rapidly “circle” things on slides as you talk, and of course, don’t wave it around the room. Laser pointers also have the disadvantage that they can give away your nervousness; if you tend to have shaky hands, simply use it quickly and shut it off again.

It is often useful to set up the expectations of the audience before showing a slide. For example: “If my hypothesis is correct, I expect [blah] to be greater than [blah]. In the next slide…” CLICK “you can see that this is indeed true.” This has the effect of focusing your audience’s attention precisely where you want it to be, so they are likely to understand your talk better.

**Coping with questions**

An important part of a scientific talk is answering questions from the audience. Make sure you understand how much time you are to allow for questions at the end of your talk. It’s rude not to allow time for questions.

However, you are not obligated to answer questions during the main part of your talk. Questions can throw you off your rhythm. If it is more convenient, don’t be afraid to postpone questions until the end of the talk, and just politely say to your questioner that you would prefer to wait.

When answering a question: First, repeat the question for those that did not hear. Think before you speak. Ask for clarification if necessary. In general, a short answer is better than a long one. Be prepared for obvious questions. Many speakers prepare extra slides for questions they expect. Admit it when you don’t know the answer, and acknowledge good suggestions for further experiments or alternate interpretations. Never be rude to your questioner (“I already said that.” “If you were paying attention, you’d see that that would be impossible!”). If you do get an off-the-wall question, answer it quickly without being patronizing and move on to the next one. If a questioner persists in asking tangential questions, suggest they could discuss it with you after others have a chance.

**Exercise 1**

Your instructor will give you a copy of a published scientific paper. You will work in pairs or groups. Imagine that this is your work, and that now you are going to prepare a talk based on this paper. First, consider the following questions:

1. Chances are that the paper includes much more information than you can put into a talk. What will you include, if you talk is aimed at the following audiences?
   - A. Colleagues in the sciences, at a scientific meeting
   - B. Other students in the class that may be just learning about the concepts in the paper
   - C. The general public with no scientific background

2. Talks may be of different lengths. What would you include in your talk if it is
   - A. 5 minutes
   - B. 15 minutes
   - C. 50 minutes
Now, each pair or group should create a presentation, following your instructor’s specifications for talk duration and audience. Your instructor may have you do this part at home or in the laboratory. As well as preparing an effective talk, prepare a series of at least three slides (separate from your talk) that demonstrates what a poor talk looks like. Present both your talk and your poor-talk examples to your classmates.

3. How did the talks differ among groups? Which talk was most effective? Why?
4. Which was the worst slide? Why? Are some sins greater than others, or forgivable in some contexts?

**Exercise 2**

One good way to learn how to give an effective talk is to attend and critique other talks. Your instructor will give you ideas about other talks to attend. Some possibilities include: scientific talks given by visiting speakers in a seminar series at your institution, talks presented by students in other sections of your course or in other courses, informal talks given by graduate students and faculty, etc. If none of these options are available, you might critique lectures given in your other classes, although classroom lectures are substantially different in format and many of the rules might not apply.

Before you go to a talk, prepare a list of items that you will note during the talk. Here are some suggestions:

- Organizational structure
- Capturing attention with the “big picture”
- Clarity of methods
- Clarity of data presentation
- Strength of conclusions
- Style, mannerisms, etc.
- Level appropriate to the audience

Compare your notes with those of a friend. Did you like and dislike the same aspects?

When you begin attending public talks, you will notice several things. First, you may possibly be surprised by the variability in talk quality. Some talks on potentially dull topics are riveting and informative, whereas others on fascinating topics are positively soporific. Second, you will notice that there are really great talks that violate many of the principles we suggest! This illustrates our final point that there are many ways to give a fine talk: the guidelines we offer are only guidelines.
Exercise 13: Participating in a Poster Session

Catherine Hackett Renner and Michael J. Renner

Goals
1. To learn how to prepare a poster presentation of a research project
2. To gain experience presenting research in a public forum
3. To gain experience answering questions about research projects

Background
Once you have conducted a study, you have an ethical responsibility to make the results of the study known to the scientific community or the public. Presenting your research in a public forum allows you to meet this responsibility.

This public presentation of your research can take several different formats. If you look at programs from conferences you will find oral (also called platform) presentations, symposia, roundtable discussions, and poster presentations. Each of these has its own unique and important role in a conference presentation. Here we will focus on the poster presentation.

The role of the poster presentation is to allow researchers the opportunity to present research that has been completed, but in a less formal setting than an oral presentation. In a poster session, presenters are in one room with their posters attached to large boards. The poster is identified by a number and the titles. Authors and abstract of posters being presented for a set period of time (usually about two hours) are listed in the conference program. Conference attendees then come to the room during this time to view a particular poster (or posters). The presenter also brings handouts describing the study; these are provided to anyone who is interested in the research. The setting is more relaxed than a paper presentation, because the presenter is typically discussing his or her poster one-on-one with individuals interested in the research rather than in a group format.

Poster presentations are a great way to begin the process of learning how to present research with a low amount of stress or anxiety. A poster presentation also can build confidence in one’s ability to conduct research. All in all, it is a win-win experience.

Methods
To get started you will need to find out how you will be displaying your poster. If you will be tacking your poster to a bulletin board, you can print each section on a separate sheet of paper. If tables will be set up for you, you will need a poster presentation board, similar to ones you used in your high school science classes. These presentation boards can be found at any office supply store; your university or college bookstore may also carry them.

There are several sections to a poster presentation. We will discuss each section separately and describe what information each section contains as well the mechanics (e.g., type and font size) for preparing the information for the poster presentation.

When submitting a work for publication, it is extremely important to follow exactly the format and style that the journal decrees: otherwise your paper will be returned without review. There is often more flexibility in poster design, but even this varies
across subdisciplines within animal behavior, and even among conferences. Your instructor will tell you if you are to follow any particular format. All posters, however, should have the following components.

**Title:** You will need to create a page displaying the title of the research. This page should also list the name(s) of the author(s) and their affiliation (that’s you and the name of your school). Posters are intended to be read from a distance, therefore the font size for all the text needs to be large enough for someone to be able to read it at a distance of 2–3 feet. Your title, however, is what will attract a person to your poster. You want the font size of your title to be larger than that of the rest of the sections. We recommend a font size of 40 points for this page.

**Abstract:** The abstract should be a brief summary of the study. The abstract should include a statement of the research problem, 2–3 sentences describing the participants/subjects, 4–6 sentences describing the methods and procedure, and 2–3 sentences summarizing the results and conclusions. We recommend a font size of 28–32 points for the title for this section and a font size of 24–28 points for the information that forms content of this section. If your poster space is limited, this section could be considered optional.

**Introduction:** You will need to provide a brief and concise summary of the research literature on the topic. You can assume that the person reading the poster has some knowledge in the area and more than likely is familiar with the background research in the area. The introduction here then serves the purpose of providing an explanation of how your study fits into this area of research, rather than educating the person about all past research in the area. This introduction does not need to be as long as one for a paper for a class or as long as one you would find in a published journal article. Two poster pages is more than likely enough to convey the necessary information. We recommend a font size of 28–32 points for the title of this section and a font size of 24–28 points for the information that forms content of this section.

**Methods:** This section will contain information about Subjects, Materials, and Procedure, often separated into different subsections. It is important that you keep the methods section as complete as possible. The person reading this section should be able to re-create exactly what you did in your experiment without having to ask for more information or clarification. However, it is also important to be concise. Unlike a scientific paper, the methods do not have to be in paragraph form. The use of diagrams, bulleted lists, and flow charts, rather than lengthy paragraphs, are well-suited for the poster format.

One way to add a depth of information without lengthening the content of your materials subsection is to include illustrations or photographs of materials you used in your study. For example, if your research used particular types of equipment you may want to photograph the equipment and use the photo on your poster (we recommend the photo be in color and at least 5 × 7 inches). If you used specialized equipment, you might want to consider including a photo of someone demonstrating how the equipment was
used. Or you might include a photo of the species you studied. Photos are not only informative but they also attract people to your poster!

As in previous sections, we recommend a font size of 28–32 points for the title for this section and a font size of 24–28 points for the information that forms content of this section. Illustrations that are not photographs should be black and white, drawn in a clear, crisp, and uncluttered fashion and fill an 8.5 × 11 inch page.

**Results:** A paragraph indicating the type of statistical analyses performed and which results were statistically significant is necessary in this section. Reporting of means, sample sizes, and standard deviations are also necessary pieces of information but may be best presented in a table or graph. Reading a table, rather than prose, is often easier on the conference attendee. Each table should fill one page. The font size can be adjusted so to make this possible. However, for readability purposes, it would be best to not go smaller than 18 point font.

**Discussion and/or conclusion:** Here is where the fun begins! Here, you can begin to talk about the meaning of your results and the relationship your study has to the published research. If you are aware of weaknesses or confounds in your study, this would be a good place to inform the reader. However, do not make the mistake of many beginning researchers and devote your entire discussion to problems with your study. This is also the place for you to mention what the next step in the research process would be and if you have intentions of pursuing future research in this area.

A final note: Posters need to be very user-friendly. Short paragraphs or bulleted lists are easier to read than long, dense passages. If you are presenting the results from more than one study, you might present the Methods and Results for each separately, grouped together in a visually appealing way. You should have a discussion section after each study and a conclusion section to integrate the outcome of both studies. We recommend a font size of 28–32 points for the title for this section and a font size of 24–28 points for the information that forms the content of this section.

**References:** Any citations in the body of the paper should be included here, in the style your instructor suggests. If you have prepared a handout (see below), this section could be excluded if you are running short on poster space. We recommend a font size of 28–32 points for the title for this section and a font size of 24–28 points for the information that forms content of this section.

**Handout:** It is becoming more common for conference attendees expect to be able to obtain a handout of your study. In making a handout you want to be sure the content of your handout is consistent with the content of your poster. It is usually possible to reduce the contents of the poster to the standard 10–12 point font and easily turn it into a successful handout. You may need to adjust page breaks and margins in order to make the handout look professional, but we encourage you to take the time to do this. We recommend that you take 20–25 copies of your handout with you. If you run out, be prepared to have a pad of paper and pen handy so that people can give you their address if they would like you to send them a handout after the conference. Good professional
etiquette suggests that you mail a copy of the handout to those who have requested it within two weeks of the conference.

Questions and/or report instructions
Your instructor will assign one or more of the following.
1. A great way to practice putting together a poster is to find a published research article and either actually create a poster presentation for it, or write a discussion of how you would do so. If it is a long paper, you may decide to present only part of it. How will you choose which section? Figures and tables that are fine in a journal might not be suitable for viewing from 3 feet away in a crowded room. How will you modify these to make them more appropriate for a poster? How will you distill the different sections of the paper down to their most important points?
2. Attending conferences is another way to learn about the most current and exciting topics in your field. It is also a good way to meet other researchers working in your interest area. If you are able to attend a conference, go to several poster sessions and critique 3–5 posters, given the above guidelines. What would you have changed to make the poster more informative or readable? Try to identify several posters that were done well and make notes about how they were constructed.
3. If you have a poster session of independent research projects in your class, formulate two questions for each poster and ask these of the author. This will give you practice at critiquing research as well as answering questions about your research that others pose to you.
SECTION 2: PRACTICING RESEARCH SKILLS
Exercise 14: Neurotransmitters and Aggressive Behavior in Crayfish

Kimberley A. Phillips, Lisa M. Shauver Goodchild, Larry N. Reinking, and David A. Zegers

Goals
1. To investigate how octopamine affects aggressive motivation in crayfish
2. To generate and test a hypothesis about how octopamine will influence the aggressive behavior of dominant and subordinate crayfish
3. To discuss the importance of blind experimental procedures
4. To develop skills in analyzing data

Background
As your textbook describes, animals often fight over valuable resources. However, instead of having numerous and potentially energetically costly and dangerous fights, many species form dominance hierarchies: submissive animals relinquish resources to dominant animals with little, if any, overt aggression.

Crayfish compete for shelter and food. Dominance hierarchies are known to form among some crayfish groups (Bovbjerg 1953, Copp 1986). Social status is created by a series of agonistic interactions that are affected by the animal’s past fighting experience (Rubenstein and Hazlett 1973). A typical agonistic interaction follows the pattern described by Bruski and Dunham (1987). One crayfish will be approached by another to begin the interaction. This is followed by “meral spread” threat displays, where the crayfish will raise their chelae anteriorly, spread the chelae apart from one another and open the claws. They may escalate the fight by coming closer to one another and begin to strike each other with the chelae. They may advance further by “interlocking chelae” and “pushing” one another. This pattern of pushing with chelae interlocked will repeat until an individual loses the fight. The loser can be identified by either of two stereotyped subordinate behaviors: a tailflip, or retreating from the other by walking away with its chelae closed and touching the substrate with its body low and elongated relative to the substrate. Dominance is often communicated to in the group often through a “flexed posture,” where the individual stands on the tips of its legs and its tail is stretched out with the tip of the tail bent. Crayfish recognize dominant states in each other via chemical cues, such as urine (Zulandt Schneider et al. 1999) or visual cues, such as chelae displays (Rubenstein and Hazlett 1973).

The proximate mechanisms of social status are neural and hormonal changes. Serotonin (5-hydroxytryptamine creatinine sulfate complex, or 5-HT) is involved in aggression in crayfish. In crayfish, as with other invertebrates, increased levels of serotonin increase levels of aggression (Kravitz 1988). Serotonin alters some of the dynamics of fighting behavior, including the likelihood of retreat and duration of the interaction (Huber et al. 1997). Yeh et al. (1996) explain that the direction of change (decrease or increase) in fight behavior is dependent on the individual’s past fight history and current social status. For example, the main nerve associated with retreat via tailflip, the lateral giant (LG) neuron, of a dominant individual will fire more frequently after a
serotonin bath is applied. After such experimental manipulation, the dominant would be predicted to be more apt to tailflip than before, ending the fight bout sooner. The opposite effect is seen among subordinates, where an increase in serotonin levels renews motivation to fight. Serotonin inhibits the LG neuron among these subordinate individuals, causing a decrease in the likelihood of tailflip retreat.

To add another layer of complexity, the past social position of crayfish also influences the effect of serotonin on the tailflip response. Crayfish that have had a change in social status—from either previously dominant to presently subordinate, or the reverse—are more likely to tailflip when serotonin is applied (Yeh et al. 1997). It is believed that this pattern arises due to a changing threshold of the LG neuron and changes in the two or three types of 5-HT receptors (Yeh et al. 1996).

**Prediction formulation**

What Yeh et al. (1996) and Huber et al. (1997) did not investigate in these studies is how the neurotransmitter octopamine may be involved in aggressive behavior. Octopamine is a serotonin antagonist, which means that it contributes to the inhibition of the receptor site and makes serotonin less effective. Kravitz’s (1988) research has shown that octopamine appears to have the opposite effect of serotonin on aggressive behavior. Given what we know about how serotonin influences aggressive behavior, answer the following:

1. What effect might we reasonably expect octopamine to have on aggressive behavior in general?
2. How might social status influence how an individual responds to octopamine? Based on the background information, generate two predictions concerning the effect octopamine will have on each agonistic behavior of dominant and subordinate crayfish.

**Methods**

For this experiment, a resident crayfish needs to be established in a testing arena. After this crayfish is a “resident,” you will need to determine whether it is dominant or subordinate. This can be accomplished by presenting the resident with a threat stimulus and recording its responses to the threat. After you have determined the status of the resident crayfish, you will test it against an intruder crayfish and record its behavior. The resident crayfish will then be injected with either saline or octopamine, and tested again against an intruder crayfish. In this way you will be able to test your hypothesis concerning the effect of octopamine and social status on the aggressive behavior of crayfish.

Place “aged” water to a depth of 16–18 cm in the testing arena and in the two small holding chambers. Select three crayfish from the larger holding chambers. Match them for sex and length to the nearest centimeter. Record the sex and length of the crayfish on the data sheet. Dry the cephalothorax of one crayfish and mark the carapace with white correction fluid. Establish this crayfish as the arena “resident” by allowing it to become adjusted to the arena for 5 minutes. Keep the others separately in the two small holding chambers. During this 5-minute period, place a paper tent over the arena and adjust the
tent until the crayfish is visible through the observation flap. Secure the tent to the arena with tape. The paper tent is simply a piece of paper folded in half and placed across the top of the testing arena. The paper tent provides a bit of shelter for your crayfish, so it is not disturbed by various movements in the classroom. During this adjustment period and for the following observation periods, you must minimize movement, vibrations, and sounds around the experimental chambers.

After the 5-minute adjustment period, present a threat stimulus to the resident crayfish. The threat consists of a #10 rubber stopper on the end of a dissection probe. Starting at about 60 cm (~ 2 feet) from the animal, approach the anterior of the crayfish with this threat. Move the rubber stopper over this distance to the count of “craay-fish” (about one second). Count the number of tail flips that occur as the crayfish escapes from the threat.

Use the behavioral responses to the threat stimulus to classify your resident crayfish as dominant or subordinate (Bruski and Dunham 1987). Those crayfish that retreat very quickly are likely subordinate. Crayfish that remain still for a period of time or move toward the threat stimulus are dominant. It is likely that your crayfish will be subordinate, due to its social housing situation prior to this exercise (think about this: why would most be subordinate?)

Add one of your other crayfish (intruder #1) to the test arena and use instantaneous sampling to record the behavior of the resident crayfish. Crayfish engage in several different behavior patterns during an agonistic interaction. These include:

**Moving toward** the intruder (a dominant response)
**Threat**, in which the crayfish does not move but stands with chelae raised and spread (a dominant response)
**Fighting**, in which the two crayfish interlock chelae and push one another (a dominant response)
**Moving away** from the intruder (a submissive response)
**Tail extended** with body close to ground, no movement (a submissive response)
**Tail flexed** while standing on tips of legs, no movement (a dominant response)

In terms of increasing degrees of escalation, moving toward an intruder is initiating an aggressive encounter, followed by threat, and then fighting. Moving away is considered a submissive response. Tail flexed and tail extended are postures that possibly indicate dominant or subordinate status of the crayfish.

Every 10 seconds for 5 minutes record the behavior of the resident crayfish using the behavioral categories above. On the data sheet in the Excel file, place a tally mark in the one appropriate box at each 10 second time interval. At the end of the observation period, remove both crayfish. Return intruder #1 to its small holding chamber. Bring the resident to your instructor for an injection. Your crayfish will be injected in the ventral sinus with either crayfish saline (.1 ml) or with octopamine (2mg; 20mg/ml of saline, inject .1ml). You will not be told of the type of injection your crayfish receives.

Drain and rinse the test arenas with hot water for at least one minute to remove any chemicals left behind by the previous crayfish. Place fresh “aged” water to a depth of 16–
18 cm in the arena. Return the resident to the arena and reposition the paper tent. Wait for a 5-minute adjustment period. Present a threat stimulus to the resident crayfish and count the number of tail flips. Add intruder #2 to the arena and observe the resident’s behavior for 5 minutes. Record your observations on the data sheet. When complete, move the resident and intruders to the appropriate recovery tanks.

Questions
1. Why didn’t your instructor tell you which type of injection your crayfish received?
2. What were your hypotheses and predictions for this experiment? Which behaviors did you predict would increase and which would decrease given the social status of your crayfish and the type of injection it received (saline or octopamine)? Why?
3. Consider the case in which your prediction is not met. Does this mean your hypothesis is not true? Why or why not? If your predictions are met, does this mean that your hypothesis has been proven true? Why or why not?
4. Before proceeding with statistical analyses, graph the frequencies for each behavior by social status for both pre- and post-injection with octopamine. Visually inspect your data. What patterns seem to emerge?
5. Which statistical test would be appropriate for analyzing your data? If your instructor suggests it, analyze your data.

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Literature cited


Exercise 15: Path Integration in Humans

Mitchell B. Baker

Goals
1. To learn about dead-reckoning and navigation
2. To make and test predictions about orientation and using landmarks
3. To learn the basics of analyzing circular data

Background
All animals that forage or travel away from a nest, burrow, territory, or other central location face the challenge of returning home. Some animals can lay a chemical trail to mark their return journey. Others can follow a sequence of landmarks or use cues directly emanating from home to find their way. Many animals do not have the luxury of landmarks or trails. The navigational feats that animals are able to perform without landmarks are astonishing. As seen in Exercise 5 of your textbook, seabirds regularly forage thousands of kilometers from isolated island colonies, though there is some suggestion that olfactory cues could provide some landscape information (Nevitt 2000), and a desert ant of the genus *Cataglyphis* can travel hundreds of meters on a foraging trip, ending up on average 25 meters from home by the time it finds a seed (Wehner 1992). The ant then travels directly back to its home colony, without retracing its steps. Even those who can use landmarks may find it more efficient to return directly home from an excursion rather than retracing a twisting, indirect, outward path.

How do animals find their way when not using trails or landmarks? Many animals perform what is known as “dead reckoning,” or path integration, where an estimate of the distance and direction home is constantly updated with each outbound movement (Etienne et al. 1998). This is what navigators at sea had to do before satellite systems and other modern navigational tools: they would take frequent measures of the speed and direction traveled, and use trigonometry to sum the distances and directions of each leg, or vector, of their journey. A vector is the line between two points, and can be represented as a distance and direction of one point to another, or by the x–y coordinates of each point. The sum of all the smaller vectors traveled is a single vector that is the estimated distance and direction home. It is important to realize that both animal and human brains integrate smaller movements into a single estimate of position without consciously working through all the mathematical calculations. When people are given a virtual reality maze to navigate (as described in Exercise 5 [Alcock 2005]) we don’t count our steps and calculate sines and cosines to find our way to a goal. On an unconscious level, however, we do use some kind of algorithm, a series of steps or calculations, that is felt consciously as a sense that home is in a certain direction, and that some turns are going to point us closer or farther from that goal. In this lab we will use humans as subjects to test how accurate those algorithms are, whether they are not just subject to random errors but also biased in favor of some directions more than others, and whether we use landmarks other than obvious visual cues to navigate.

The simplest test for path integration and an internal map is to displace an animal moving towards a goal and observe its movement. What would happen if an animal
relying on landmarks (which can be chemical, visual, or based on another sense) were displaced to a new area without the familiar landmarks? It would start to search for the missing landmarks, perhaps circling or doubling back in the area where it was released. When *Cataglyphis* ants are displaced from a remote location, they are not confused. Instead they walk in a straight line to where home would have been had they not been displaced (Wehner 1982). However, ants, as well as other animals, do not perform perfectly in displacement tests, and we can use the errors animals make to understand how the animals are making their calculation. When forced to travel a simple two-leg maze and then allowed to orient towards home, several arthropod and mammal species show a consistent bias in their estimated return direction (Ettiene et al. 1998, Wehner 1996). They consistently choose a greater return angle (biased towards the outbound path) than the actual home direction. Vector summation does not lead to any bias in homeward orientation, but some possibly simpler methods of averaging path vectors result in biases similar to those observed in animal navigators. These biases may reflect a constraint on animals’ ability to represent inside their minds the distance and direction home, but some have argued that the biases seen in animals might be a helpful adaptation (Hartmann and Wehner 1995).

We have seen how human navigators at sea find their way, but what about humans without calculators? It’s clear that people can visualize complex maps, but it isn’t known how much people rely on those internal maps, or how much they unconsciously integrate paths in a manner similar to other animals. In this lab you are going to use human subjects to explore path integration and test hypotheses about navigation. You are going to use the failures in navigation to answer questions about how humans perform intuitive path integration. You are also going to manipulate some potential landmarks that humans may unconsciously use to orient and navigate.

**Hypothesis and prediction formulation**

The general approach is to guide subjects in simple two-leg paths under varying conditions, and record the subjects’ best guesses about the direction to the starting point relative to the actual direction. You may address one of the following questions as a class, or you may work through some or all of them in small groups. Even though you will likely split up into small groups to collect data, you will increase your sample size and greatly increase your chances of obtaining statistically significant results if you collect data that can be combined with those of other groups to ask one or two questions. You may decide that you need to bring special equipment to the testing area.

**A. Do humans use multiple cues?**

The first general question is under what conditions can humans use path integration as opposed to direct orientation, or reference to landmarks. Certainly there are times when orientation in the dark is necessary. Movement in habitats where landmarks are not distinct, such as in unfamiliar areas or in the dark, would be greatly aided by the ability to integrate paths. The ability of humans (and other animals) to use multiple cues to navigate makes it tricky to nail down the effects of any one system. Let’s consider a blindfolded human. If the subject were made to walk 20 meters in one direction, make a right turn, and walk 10 more meters, how would they know in what direction home is? They might
use path integration: they might keep track of their internal impressions of the turns and steps taken to update an estimate of the homeward direction. But there are some other possibilities. A landmark does not have to be visual. What other senses could be used to detect reliable indicators of position (landmarks)? How could you test for the use of this alternative landmark?

There are three ways to test for the use of an alternative navigational system: masking the system, enhancing the system, or creating a conflict with the alternative system. We are going to carry out an experiment where humans are led through a simple two-step path and asked to point to where home is. The subjects will be blindfolded, because at this spatial scale any of us would simply look around for the place where we started, without using any other navigational ability. By masking visual cues we are forcing the subjects to use other ones. If we were interested in whether or not people use direct visual cues to navigate, we would compare how people with and without blindfolds recognized where they started from, but this wouldn’t be that interesting. How could you mask, enhance, or create conflict using your alternative landmark system? Decide which alternative landmark system you will test for, and decide which equipment you will need to bring with you to the testing area.

B. Bias in the path integration system

The second general question, assuming that people are able to perform path integration, is whether there is any bias in our path integration algorithm. There are going to be errors in any act of navigation, which will be reflected in a spread of the directions home guessed by the subjects, but on average, an unbiased path integration system would point towards home. If, however, the system is biased in one direction or another, the average of the estimated directions home will be significantly different than the true direction. The bias can either be towards or away from the outbound path. In Figure 1 the error is scored as positive because it is pointed towards, rather than away, from the outbound path. In the Analysis section you will learn how the mean direction chosen by your subjects is calculated, and how to test whether there is a bias in the subjects’ path integration. In your groups, before you go out, consider possible sources of bias.

Methods

Your instructor will identify an open area for your experiment in an area where there will be plenty of passers-by who can be recruited to provide your data. It is a good idea to start collecting data on the members of your group for practice, but to test hypotheses you will want to use subjects who are less familiar with the shape of the maze than you are, and you want your data points to be independent, so each one should be from a different individual. You will now set up your experiment as in Figure 2. Mark the origin and the turning point at a distance 10–20 meters away (the outbound leg). The longer distance is better because it simplifies measurement of direction, and because it increases the magnitude of navigation errors. At the turning point, determine the bearings 120° to the left and right of the outbound path, and mark the final locations at half the distance of the outbound path (the turn legs). It is useful to have outward legs to the right and left of the outbound leg, especially if you have room for only one or two mazes and want to run more than one student at once. Use a protractor and two straight edges to
measure the angles, or better yet, use trigonometry. What is the length of the return leg (the distance home from the end of the turn leg), given that you know the length of the two other sides of the triangle and the opposite angle? If you stretch two tape measures from each end of the long leg, one of half the length of the long leg, and the other of the calculated distance, they will meet at a point that forms a 120° angle with the outbound leg.

To collect a data point, blindfold the subject and have a “chaperone” guide the subject to the origin. Tell the subject that they are at the origin and guide her or him to the turning point. Turn the subject and walk them to the end of the turn leg. At this point, the chaperone steps away and asks the subject to rotate themselves and point towards the origin. The chaperone or someone else measures the error, defined as the angle between where the subject is pointing and the true direction towards the origin. Record the error angle as positive if it is towards the outbound leg and negative if it is away from the outbound leg.

**Analysis**

*The challenge of circular data:* Circular data, like directional orientation or time of day, present challenges to analysis that distinguish them from linear variables like speed, voltage, or weight. For example, what is the average of 2200 hours and 0600 hours the next day? Intuitively it is 0200 hours, but how do you represent that mathematically? What’s the average of 330° and 50°? The arithmetic mean is $(330 + 50)/2 = 190$. This is clearly problematic (think of an analog clock: is 7:00 PM halfway between 12:30 PM and 1:30 PM?). The intuitive solution is to restate 330° as $-30°$ (or restate 50° as 380°), yielding an intuitive mean direction of 10°. You can carry out these corrections for pairs of directions, but it turns out that it is impossible to develop consistent rules for adding or subtracting 360 when you want the mean of more than two angles.

Statisticians have developed a solution for this problem, which will be described briefly here (all analyses used in this lab are described fully in Batschelet 1983, and further treatment of circular statistics is found in Fisher 1993). The solution is to convert each directional data point into a vector of length one, called a unit vector, with a direction equal to the observed orientation. The reason this is helpful is that vectors can be added to each other, and the average direction is then the same as the direction of the sum of all the vectors. Each vector can be broken down into x and y components using trigonometry ($x = \cos \phi_i$, $y = \sin \phi_i$, $\phi_i$ is an angle formed between the vector and the x-axis). The x and y components can be summed, and their sums divided by the number of data points will yield the average x and y displacements (how an individual moved) for the set of orientation angles. This average displacement can then be converted back into vector (distance and direction) form. This procedure can be illustrated using an example. Let’s say we have two experimental groups. The first one has subjects wearing blindfolds, and we will call them the control group. The second also wears blindfolds, but has an additional acoustic landmark to potentially aid their navigation, and we will call them the experimental group. Let us answer four questions, though we may think of many more:

1. Are our control subjects randomly oriented or are they consistently choosing some direction?
2. Is there a bias in our subjects’ guesses away from the true homeward direction?
3. Is there a difference in the mean direction chosen by control and experimental subjects?

4. Are the subjects in one treatment group more or less variable in their chosen directions than those in another group?

For each of these questions, the statistic is either automatically calculated for you or calculated using the workbook provided. Below is described what the workbook is calculating or how to use it to calculate a given statistic.

**Collecting and entering data**

The first page of the worksheet entitled “datasheet” can be printed out for collecting data in the field. Let’s say that the experimental setup has an outward leg of 20 meters in length, and a turn leg of 10 meters at a 120° angle from the outward leg. The true return direction in this case is 210° relative to the outward leg, as in Figure 2. The error relative to true return path is what you measure while the subject is pointing to their guess for where they started. If the first experimental subject pointed 35° closer to the outbound path than the true homeward direction (245° relative to the outward leg), that is what you will enter, and the spreadsheet will use the errors to calculate the directions, x- and y-components, and directional statistics. There are two worksheets within the workbook for entering data, one for the control group (Control data), and another for the experimental group (Experimental data). Enter the following 7 data points collected from control treatment subjects in a copy of the orientation lab workbook: 35°, 25°, –8°, 50°, 40°, 3°, 28°. These data points are the subjects’ errors, the differences between the true direction home and the subject’s guesses. If you’ve entered the data correctly, the upper left of the Control data worksheet of the workbook should look like Figure 3a).

Before any statistical analyses take place, the data are broken down into X and Y components. For example, the first subject’s positive 35° error means that she was pointing towards 245° relative to the outward leg. A 245° unit vector has an X-component of one times the cosine of 245°, or –0.4226. The X- and Y-components of each directional data point are calculated and displayed along with the average X and Y-components of all the subjects’ guesses on the right-hand columns of the worksheet (see Figure 3b). Now that we have broken down each directional guess into x and y components, we can start to ask questions.

**1. Are our control subjects randomly oriented?**

In order to understand whether our subjects are randomly oriented or generally pointing in one direction more than others, the spreadsheet calculates the average direction and an average displacement of all the subjects. The mean displacement, \( r \), is calculated using Pythagorean Theorem. In this example,

\[
\bar{x} \quad \text{(the average value of } x \text{)} = -0.544 \\
\bar{y} \quad \text{(the average value of } y \text{)} = -0.774
\]

so

\[ r = 0.946. \]

Why do we care about \( r \)? Imagine subjects randomly oriented. If we moved one step in each direction chosen by the subjects, on average we’d have a balancing amount of movements in all directions, and we’d end up close to where we started. This is reflected in an \( r \) close to 0. On the other hand, if each subject pointed in exactly the same direction,
then if we moved one step for each direction chosen, on average we’d be moving one unit
away from our starting point each move. An $r$ close to one indicates that all the data point
consistently in one direction, while an $r$ close to zero indicates that the subjects are not
consistently oriented in any direction. Using $r$, we are ready to answer the first question,
whether or not the subjects were randomly oriented. A simple test, the Rayleigh test, is
used to determine if the subjects are significantly oriented in any direction, or whether
their orientation is indistinguishable from random. The statistic, $Z$, is $N \times r^2$, where $N$ is
the number of subjects, and is calculated in column L of the data analysis spreadsheet.
The calculated $Z$ is compared with the critical value for significance at a $P = 0.05$ of
approximately 2.97 (Batschelet 1981), or the precise values generated in the spreadsheet.
In this case $Z = 7 \times 0.946^2$, or 6.264, which is greater than the critical value for 0.05 of
2.447 (Critical $t$ in the worksheet) so we conclude that the subjects are significantly
oriented in some direction.

2. Is there a bias in our subjects’ guesses away from the true homeward direction?

A bias in subjects’ guesses would suggest that humans are not using vector
summation to integrate paths, and would also force us to ask why the bias exists: does it
show an inherent weakness in our ability to navigate, or is there a reason for having a
bias? We will test for a bias by estimating a confidence interval around our estimate of
mean orientation, and seeing whether or not the true direction home falls within that
interval. A confidence interval is a range of values that has a specified probability of
containing the parameter being estimated. In other words, we estimated our subjects’
orientation, but errors in recording data, variation among subjects, and the limited number
of subjects recorded means that the true orientation of the subjects is likely different from
the mean of our observations. If the actual direction home falls within the range of the
observed mean plus or minus the confidence interval, we must conclude that there is no
evidence of a bias, a significant difference between the subjects’ chosen directions and
the true direction home. If, however, the true direction home falls outside the confidence
interval around our subjects’ mean chosen direction, we will conclude there is a bias in
their orientation.

First we must know the mean direction chosen by the subjects, and then we can
estimate the confidence interval around that mean. The worksheet uses the $\bar{x}$ and $\bar{y}$
values calculated above to estimate the mean subject direction. The tangent of an angle
formed by a line and the x-axis is $x/y$, so the arctan function is used to estimate the angle
of the subjects’ orientation from $\bar{x}$ and $\bar{y}$. If $\bar{x}$ is positive, the mean orientation angle is

$$\text{arctan}(\frac{\bar{y}}{\bar{x}}).$$

If $\bar{x}$ is negative then the orientation angle is $180^\circ + \text{arctan}(\frac{\bar{y}}{\bar{x}})$. In our
example $\bar{x}$ is negative, so the mean angle is $180 + \text{arctan}(-0.544/-0.774) = 235^\circ$. Now
that we have the mean orientation angle we want to know the range above and below that
mean where we have at least some degree of certainty (usually 95 percent) that the true
mean actually lies. This is called a confidence interval. If the true direction home falls
outside the confidence interval around the mean of the subjects’ guesses, we will
conclude there is a significant difference between the guesses and the true homeward
direction.
The confidence interval is calculated by the worksheet as \( t(\alpha) \times s/\sqrt{n} \), where \( t \) is a standard distribution, \( \alpha \) is 1 minus the desired \( P \) value, usually 0.95 or 0.99, \( N \) is the number of subjects, and \( s = 180/\pi \times 2 \times (1-r) \times 1/2 \). For our example, for \( \alpha \) of 0.95 \( t \) is 2.78 for our sample size \( N \) of five subjects. Using the \( r = 0.946 \) the spreadsheet calculated for our sample, \( s = 18.84^\circ \), and so the confidence interval is \( \pm 17.4^\circ \). Because the subjects’ mean orientation of 235° is 25° greater than the true homeward direction of 210°, we conclude that there is a significant bias in their homeward direction.

We have used the tests described above to ask whether our subjects were oriented randomly, or whether their orientation is significantly different from the true home direction, but what if you collect data from a second group of subjects that have been treated in ways that might make them better or less well oriented than the first group? We are now ready to compare the orientation of different groups, and two analyses used to compare orientation in different groups are calculated in the Control vs. experimental worksheet.

3. Is there a difference in the mean direction chosen by control and experimental subjects?

The Watson-Williams test is a powerful method to determine whether two groups are oriented in significantly different directions (Batschelet 1983). The test uses what are called resultant vectors to determine whether two groups are oriented in the same or different directions. A resultant vector (\( R \)) is the sum of all the unit vectors (described above) for a single population. It is also the same as \( N \times r \), where \( N \) is the sample size and \( r \) is the mean displacement as calculated above. What the test basically does is compare the sum of the lengths of the \( R \)’s for control and experimental groups with the \( R \) calculated for both groups data combined. Let’s say the two groups were oriented in exactly the same direction. In this case the experimental group’s resultant vector (\( R_e \)) plus the control group’s resultant vector (\( R_c \)) would be exactly equal to the resultant vector formed by summing the X and Y components of the two groups combined (\( R \)). If, however, the two groups are oriented in different directions, the combined \( R \) would be smaller than \( R_c + R_e \), and the more different the directions the two groups are oriented towards, the greater this difference. The Watson-Williams test uses these values and the sample size and concentration parameters for each group to calculate a test statistic that is distributed similarly to the \( F \) distribution, and the value of \( F \) and its statistical significance are calculated in the Control vs. experimental worksheet.

In our example, we have added an acoustic landmark cue in our experimental group. Enter the following values in the Experimental data worksheet; 10, –6, –8, 15, 5, 7. If the data were entered correctly, \( r \) for the experimental group is 0.9909. What does is mean that \( r \) is much higher than in the control group?

4. Are the subjects in one treatment group more or less variable in their chosen directions than those in another group?

The Watson-Williams test is powerful, but assumes that the variability in chosen directions is similar in the two groups. In addition, if one group is more confused than another, this might not lead to a different mean orientation, but might lead to a greater spread or angular dispersion than in the other group. It is an interesting question when we
might expect to see one effect or the other. A parametric test using the $F$ statistic can be performed to look for differences in angular dispersion. The $F$ value in this test is calculated by the worksheet as follows:

$$F = \frac{(n_2-1)(n_1-R_1)}{(n_1-1)(n_2-R_2)} \quad \text{or} \quad F' = \frac{1}{F}$$

whichever ($F$ or $F'$) is greater than 1, where $n_i$ and $R_i$ are the sample sizes and resultant vectors for the control and experimental groups. $F$ (or $F'$) is then compared to the critical values for the appropriate degrees of freedom ($n_i-1$) and desired significance level with tables found in any statistics text. If $F$ (or $F'$) is greater than the critical value, the two groups significantly vary in their dispersion.

**Questions**

After collecting the data, you will return to the lab and combine each group’s data into separate workbooks for each experimental treatment.

1. What was the landmark cue you decided to test? How did manipulate that landmark cue?
2. Mark the angles chosen by the subjects in Figure 4, calculated in the workbook by adding the errors to the true homeward direction (210°). Make marks at the appropriate angles around the edge of the circle, using different symbols for the blindfold-only treatment and the blindfold + landmark manipulation treatments.
3. Is either group randomly oriented? Show the appropriate test statistic, calculated from the spreadsheet and compared to the critical value for $P = 0.05$.
4. Was there evidence of use of this landmark? What was your null and alternative hypothesis? What statistic did you use? What was the value of the statistic and can you reject your null hypothesis?
5. Is there evidence of a bias in the homeward direction chosen by subjects? Test this for both blindfold-only and blindfold + landmark groups. What were your null and alternative hypothesis? What statistic did you use? What was the value of the statistic and can you reject your null hypothesis? Were the results consistent in both groups?
6. Hartmann and Wehner (1995) wrote a neural network model (a computer model that simulates the activity of collections of neurons) of path integration and concluded that on a neural level it might not be any “harder” to create an unbiased path-integration system than a biased one (the two methods can be carried out with a similar number and arrangement of circuits). They suggest that the errors seen in many animals might be adaptive. What might an adaptive function be for the bias in turning angles (to make a sharper turn than the true direction home) chosen by insects, spider, rats, dogs, and (maybe?) humans? *Hint:* Remember that use of path integration doesn’t exclude the use of other navigation systems. How would the observed bias make it more likely that other cues could be used to return home?

**Literature cited**


Figures

Figure 16.1 A two-leg maze with a turn angle of 120° where the turn leg is half the length of the outward leg, resulting in a return direction of 210° (assigning the outward direction as 0°, the angle from that direction to the true direction home is the return direction). The average of the 7 bearings are 15° towards the outbound path from the true return direction (errors leading towards the outbound path are counted as negative).
Figure 16.2 A generalized 2-leg maze with turn legs in both directions so subjects can be led in either direction.
Figure 16.3  

**a.** The data as entered in the accompanying workbook in either the experimental or control worksheets. **b.** The calculated directions and x- and y-components of each subjects’ guess.
Figure 16.4 The return directions (actual and subjects’ guesses) are scored in relation to the outward leg; the direction of the outward leg is 0°.
Exercise 16: Number vs. Body Size of Offspring in Biparental Burying Beetles
Stephen T. Trumbo, Garrison Smith, and Derek S. Sikes

Goals
1. To generate and test a hypothesis about the relationship between number of offspring and body size of offspring that parents can rear
2. To predict when parents might adjust brood size to different environmental conditions, and how that adjustment might be made
3. To understand the difference between predictions made about data variation within a treatment, and data variation between treatments
4. To predict the relationship between the size of parents and the size of their offspring

Background
Every aspect of behavior, physiology, and morphology involves tradeoffs. Organisms clearly cannot be designed to do everything maximally. Organisms designed for large body size are not the quickest, organisms designed to withstand cold temperatures may be more likely to succumb to heat.

One of the fundamental trade-offs in reproductive biology is the trade-off between the number and size of offspring (Smith and Fretwell 1974). Behavioral ecologists believe that the details of this trade-off are under the influence of natural selection (Hendry et al. 2001). Some species produce fewer, heavier young because large individuals may be more competitive for resources or mates, or more fecund. Other species produce many, small offspring because smaller individuals may develop faster, or have lower nutritional demands, or simply because more offspring means more individuals seeking to pass on the parents’ genes in the following generation (Calder, 1984, Oksanen et al. 2003).

In addition to differences found between species, there also may be differences within a species in the number/body size trade-off. The relationship between the body size of a parent and the body size of its offspring is, in part, a direct genetic one. In species in which parents provision their young with food, however, the body size of offspring may be indirectly affected by the parents’ decision on how many young to rear. This complicates the genetic correlation between the body size of the parent and the body size of offspring (Hunt and Simmons, 2002, Rauter and Moore, 2002). Parents might be expected to adjust brood size, and therefore alter body size of offspring, if they have reliable information about either the competitive environment the offspring will face, or the amount of food available for their own offspring. If parents have information indicating a very competitive environment for offspring, their reproductive success may be enhanced by producing fewer, larger young (Strickler, 1982). If parents have information about food available to their own offspring, it may be beneficial to adjust brood size. Food resources that are easily assessed, such as carrion and dung balls, may lend themselves to brood size manipulations by parents (Hunt and Simmons, 2002; Rauter and Moore, 2002).
The organisms that you will use in this exercise manipulate a discrete resource packet, a small vertebrate carcass, for use by their young. The quantity of this resource can be assessed more easily than resources used by most animal young. You will attempt to predict the nature of the number/body size tradeoff, and to determine how burying beetle parents use information on resource quantity when making reproductive choices.

Natural History

There are 66 known species of burying beetles (*Nicrophorus*) in the world, most of which are found in North America and Eurasia. These beetles are important scavengers, integral to breaking down small vertebrate carcasses. Burying beetles emerge as adults and must spend 10–20 days feeding on carrion and carrion fly larvae before they are ready to breed. When reproducitively mature, they search for a small, fresh vertebrate carcass. A single female or a male–female pair must work quickly on this resource or they risk losing it to competitors such as ants, carrion flies, or vertebrate scavengers. The resident beetles prepare the carcass by burying, removing hair or feathers, rounding it into a ball, and depositing secretions on the surface. If the carcass is not located in a place suitable for burial, the beetles will first move the resource to a suitable spot. Eggs are laid in the soil surrounding the buried carcass in as little as 16 hours. The resulting larvae hatch out in 2–4 days (varies with temperature and species), and crawl to the carcass to be cared for by their parents. Larvae feed directly from the carcass and are also fed regurgitated liquefied carrion by their parents. These regurgitations are most important early in development. The first and second instar each last about 24 hours. The third instar lasts 5–10 days. Mature larvae disperse from the nest, work their way into the soil, and enter a pre-pupal stage. Some species overwinter as adults and some as pre-pupae.

1. When different burying beetle parents are given the same quantity of resources to work with, what will be the relationship between the number of offspring in a brood and the size of those offspring?
2. What will be the response when burying beetle parents are given more resources (a larger carcass) to work with? Will parents rear more young, heavier young, or both?
3. What will be the relationship between the size of the parents and the size of the offspring?

Methods

The procedures for setting up breeding containers and obtaining data on reproductive output are given below. If you wish to incorporate observations into the experiment, first carefully read the instructions below for Days 4–5 where techniques of observation are described.

Day 0

Before you handle the beetles, you should prepare a breeding container for each male–female pair you will use. Your instructor will suggest how many containers to prepare. For each male–female pair you need a “carcass.” The carcass may be a thawed mouse (previously frozen) or large chunks of chicken liver. Weigh each carcass and have them ready as you prepare the breeding chamber. A breeding chamber can be any
container that will hold soil to a depth of at least 10 cm, and that can be covered easily with a secure top or lid. The minimum diameter of soil in a round breeding chamber is 8 cm. Have your male–female pairs ready in small containers (these must have a tight lid since these beetles are very good at crawling or flying out). The beetles may already be marked as males and females or you may need to sex the beetles. The beetles can be sexed by careful observation of the last abdominal segments (Trumbo 1996; see Figure 1). A hand lens may be helpful in sexing beetles. If you sex the beetles yourself, clip the edge of the elytron (wing cover) on one side for the male and on the other side for the female. This will facilitate a quick determination of sex later in the experiment. Weigh the male and female parent, separately. In your breeding container, push the soil to one side, place a male and female into this depression, and cover the beetles with soil (beetles covered with soil tend to reduce their movement). Place your carcass on top of the soil. You may want to cover the carcass with a paper towel. If you are using chicken liver as a carcass, be sure not to press the paper towel into the carcass. Secure the top of your container with masking tape if the top is such that it does not snap shut. Place the containers in dim light where it will not be disturbed.

![Figure 1](image-url)

**Figure 1.** Burying beetles can be sexed by comparing their abdomens. Note the two parallel stridulatory files on the animal. (These can be seen where the wing covers terminate.) Females possess three exposed abdominal segments past the stridulatory file while males have four segments. To facilitate inspection, the abdomen can be gently pressed on the sides to force the last abdominal segment farther out.

**Days 4–5 (Making Observations—Optional)**

Burying beetles have fascinating parental behavior and you may want to take the opportunity to observe parent-offspring interactions. For observations it is best to use broad breeding containers such as a plastic shoebox. Although some parent-offspring
interactions can be observed on Day 7, most parental feedings of young occur in the first 36 h that young are on the carcass (Fetherston et al. 1994). For most species of burying beetles, the larvae will hatch out and crawl to the carcass on Days 4–5. To observe parental behavior, you first have to prepare the set-up for observation an hour or more before actual observations. To prepare a setup for observation, open the breeding containers and inspect the carcass for very small, first instar larvae. If larvae are present, gently pull the carcass to the soil surface and place the carcass in a slight depression in the soil such that the top half of the carcass sticks above the soil surface. Record the appearance of the carcass (shape, presence of hair, etc.). Replace the lid for the breeding container (or you may substitute a glass plate). Place the breeding container in a location where you wish to make subsequent observations. Observations should be made under very dim light or under a red light in an otherwise dark room. Observation containers should be placed on a secure table that is not subject to vibrations. When you are ready to make observations, gently lift the lid or glass plate. If you use a glass plate and observations can be made directly through the glass plate, you can leave it in place. Be careful not to jostle the container, as disturbance will cause the parents to hide in the soil. Be careful not to exhale on the beetles as this also causes them to temporarily desert the nest. Note the behavior of larvae when the parent is not near. Do they search for the parent? Do they beg for food like bird nestlings? What is the parent doing when not near larvae? What parental behavior immediately precedes feeding young? Do parents make any sounds during parental care? (Listen carefully). When the parent feeds larvae, are all young fed? Do you see any unexpected parent–offspring interaction?

**Day 7**

On Day 7 you need to check your breeding containers to confirm that larvae are present. Most all of the containers should have larvae by this point. If there are no larvae on the carcass, and the carcass is not rounded into a clean sphere, then the female was not ready to breed. The carcass should be discarded. To inspect the carcass you may need to dig in the soil and pull the carcass toward the surface. If the breeding container is a large cup, gently shake the contents of the breeding container into a tray, and then inspect the carcass. Record the shape and appearance of the carcass. You may want to use gloves while doing this. While inspecting the carcass, briefly transfer the male and female to a separate cup. Remove the male permanently from the breeding container and replace the female underneath the carcass. The female may run away but she will quickly return and again provide care for the brood. The male parent needs to be removed because this is near the time he will desert the nest under natural conditions. If he is confined to the nest area artificially, he may return as an intruder and disrupt nesting, even to the point of killing some of his own mature young. Place the carcass back into a depression in the soil so that the top of the carcass is even with the soil surface. You may want to cover the carcass with a paper towel if the breeding containers are being kept in an area where significant light gets into the container.

*(Optional)* Interesting parental behavior can often be observed on Day 7. If you wish to make observations at this time, follow the instructions for observations for Days 4–5.
**Day 14**

On Day 14, all of the larvae reared on the carcass should have dispersed into the soil. Empty the contents of the breeding container into a sorting tray, place the larvae into a small cup, and remove the female. Record the appearance of the carcass. If there is a significant amount of useable material remaining (just about anything except hair and bones), make a note of this. If the larvae are relatively clean, they can be counted and weighed directly. If soil adheres to the larvae, wash the larvae (they can be safely submerged in water for up to a minute), dry on paper towels and then weigh as a brood. Handle all your replicates in the same way. Record your data on a chart provided (combine with data from your classmates if your instructor directs). You are now ready to analyze the data.

**Questions**

1. In many species, parents vary how much they invest in each individual offspring. Some parents may produce many offspring, investing less in individual young, while other parents produce fewer but larger young. What environmental conditions might favor production of a greater number of young? What environmental conditions might favor a greater investment in each individual offspring?

2. In many species, the condition of the parent(s) affects how many young are reared. How would you expect the condition of a parent (well fed/not well fed, healthy/sick, heavier mass/smaller mass) to affect the decision about the number of young to rear? What might the condition of the parent indicate about the favorability of the environment for rearing young? When is the condition of the parent more likely and less likely to be related to their success in rearing more young or heavier young?

3. At what point in the nesting cycle (before egg-laying, after egg-laying, after hatching, after young have begun to put on weight) is it best to reduce the number of young in a brood to match the available resources? What are the advantages and disadvantages of making this decision earlier versus later in the nesting cycle? What are the possible ways that burying beetles vary the number of young they rear?

4. How would you expect the reliability of information about resources to affect parental care decisions? For what kinds of resources would you expect that it would be more likely for parents to adjust their clutch or brood size?

5. To adjust brood size in an adaptive way parents require information about the number of young they have as well as the quality of resources available for their young (or information about closely correlated variables). How might species acquire this information? In what ways might burying beetles acquire this information?

**Literature cited**


**Exercise 17: Simulating the Evolution of Honest Signaling**

Paul E. Brunkow and Teresa A. Cochran

**Goals**
1. To recognize the potential ultimate (evolutionary) benefits of signaling competitive ability to prospective rivals in contests over resources
2. To recognize the heuristic value of simulating a complex process with a simple exercise
3. To identify assumptions and consequences of those assumptions underlying such simulation exercises

**Background**

In many species, a situation can arise in which two (or more) individuals will need to compete directly for some resource that is of value to them. Such resources might include food, a mate, a refuge location or a territory, and competition for these resources can take the form of aggressive behaviors directed by individuals against each other. How individuals “should” behave under such circumstances so as to maximize their reproductive success has been commonly modeled in the context of “game theory” (Maynard Smith 1982). Game theoretic models incorporate various natural historical aspects and assumptions about the biology of the species being studied, and then play various behavioral strategies against each other (using mathematical functions or computer simulations) to determine the “best” strategy for individuals to follow. These models typically offer specific hypotheses that can be tested using natural species, thus allowing us to continue to improve our understanding of the evolution of particular competitive behaviors within diverse species.

Contests between rivals are typically won by the individual who is larger, in better overall physical condition, or more experienced in such contests; in general, we say that the winner is typically the individual with the higher “Resource Holding Potential” (RHP) (Parker 1974). In “war of attrition” models, an individual is assumed to compete with another only up to a certain level, at which point it retreats from the contest. Importantly, neither individual knows how far the other is willing or able to go, and the contest simply ends when one of the contestants reaches their own maximum level of investment in the contest (Maynard Smith and Parker 1976). Contests in larval damselflies have been described as following this war-of-attrition model (Crowley et al. 1988).

Parker and Rubenstein (1981) modified the war-of-attrition model to include the ability for individuals to assess each other’s RHP. Using mathematical simulations, they found that such “assessor strategies” were evolutionarily more advantageous than non-assessor strategies: individuals that could determine how well their opponent would be able to fight for a resource relative to their own ability to fight would not waste time and energy (or get injured) engaging in contests they “knew” they would lose anyway. Thus, these assessor individuals would leave more offspring in the long run. Many more models of contest competition incorporating the ability of individuals to make decisions about whether to continue fighting have been developed, including the “sequential assessment

Deciding whether to continue fighting an opponent for a resource by following an assessor strategy requires that individuals be able to accurately detect their own RHP relative to their opponent’s RHP. It is evolutionarily costly for an individual to either (a) engage in an injurious fight with an opponent mistakenly assessed as being of lower RHP, or (b) surrender a valuable resource to an opponent mistakenly assessed as being of higher RHP. Behavioral biologists have long recognized that contest resolution through assessment of RHP is therefore subject to “bluffing” behavior (Parker and Rubenstein 1981; Rohwer 1982): an individual could gain great advantage by signaling that they possess very high RHP when in fact they do not, in the hopes that prospective rivals surrender valuable resources without actually engaging in a costly fight.

Studying the evolution of contest resolution through mutual assessment has thus included discussion of the evolution of “honest” signals (Rohwer 1982; Veiga 1993). Honest signals are those which convey to an opponent an accurate measure of RHP. Through proposed physiological pathways or actual morphological appearance, signals such as badge size in sparrows (Veiga 1993) and antler size and roaring ability in red deer (Clutton-Brock and Albon 1979) truly reflect the ability of an individual to win in a contest over a resource. If “cheaters” try to produce these signals when they do not possess the actual RHP to back them up, they lose in contests when challenged by individuals possessing similar signals.

For contest resolution through the use of honest signals to evolve in a species, individuals who make use of (and honor) such signals must experience higher reproductive success than those who do not. Honest signaling should increase reproductive success of these individuals because they will not have to waste time and energy engaging in contests they clearly will win (if they have high RHP) or clearly will lose (if they have low RHP). In this laboratory exercise, you will simulate the evolutionary benefits of honest signaling by engaging in contests over territories with your classmates. Your “reproductive success” during the exercise will be determined by how well you can maintain ownership of a territory, both under conditions where you cannot know your opponents’ RHP and under conditions where you will be able to estimate your opponents’ RHP.

**Methods**

**Basic Biology**

It is the beginning of the breeding season and you are a’ hankerin’ to set up a territory to which you may be able to attract mates. The tables in the room each represent two territories (for a total of 12 territories in the room), and the territories vary with respect to their quality as indicated by the letters on each table (type “A” quality > “B” quality > “C” quality). At the beginning of each round (breeding season), you simply go claim one of the territories in the room. If there is no one at that territory and no one challenges you to the territory, then you are free to reproduce all you want (metaphorically speaking, of course!). However, you may have to challenge an individual that has already claimed the territory or you may get challenged by another individual.
who also wants the territory. To fight off any challengers to the territory, you need to expend energy. Your success at reproducing in the territory on which you end up will be determined by how much energy you have remaining after fighting off challengers. *The goal of this game is to maximize your reproductive success in each breeding season and to insure that you stay alive to breed in the next breeding season.*

**Rules for Challenging and Energy Expenditure—Version I**

You will be allotted a certain amount of energy at the beginning of each breeding season in the form of a number written on a card; the number represents the number of “energy units” you have at that point in time. *You may not reveal this number to anyone else at any point!*

To claim a territory, simply walk to that table and stand there. If the territory you want is occupied, then you are free to choose another territory or to challenge the current occupant. Current occupants must signal their intention to dispute the territory by remaining at the territory.

To challenge an occupant, the challenger should write the number “1” on the sheet of paper in the center of the territory. This represents your investment of one unit of energy to fight off the resident.

If you are challenged as a territory holder and you have no intention of holding the territory, then you must leave the territory before the challenger “challenges” (i.e., writes a “1” on the paper). If you are challenged and you want to retain the territory, then you must enter into a fight with the challenger and write a “1” on the paper also.

If you are a challenger and the occupant has begun to fight and you still want the territory, then you must cross out your “1” and write a “2” on the paper. If you do not want to continue fighting, then you simply leave the territory and look for another one.

If you are the occupant, and the challenger has remained to continue fighting by writing a “2” and you still want to retain the territory, then you must cross out your “1” and write a “2.”

Fights continue in this fashion: each player invests one unit of energy at a time in staking their claim to a territory, each time crossing out their number and writing the next higher one on the paper. As soon as one player has decided that they do not want to expend any more energy in the fight over that particular territory, s/he leaves the table and the other player retains/claims occupancy. *In no case can any player invest more energy in a fight than they have written on their card!* If you feel yourself forced to write a number on the paper which is equal to the number on your card, that means you invested all of your energy in the fight and you are now dead! (...so don’t do that!)

Upon completion of any given territorial dispute, both players must subtract from the number on their card the units of energy invested in that fight. For example, if Player J (the occupant) started with 40 units and invested 23 units in the fight, and Player Y (the challenger) started with 30 units and invested 24 units in the fight, then Player J has lost and leaves the territory with 17 units remaining to look for another territory, and Player Y becomes the occupant with only 6 units remaining to fend off further challengers and to attempt reproduction. *Both players should write the new number of energy units they have remaining on their card before challenging for a new territory—do not let anyone*
see the number you write on your card! Also, place a clean sheet of paper on the table with no numbers written on it before another fight ensues over your territory.

Each round (breeding season) continues as such until all territories are filled and all other players have opted out of trying to secure a territory. Note that there are far fewer territories than there are individuals who want them!

At the end of each round, report to the instructor (a) how much energy you have remaining, and (b) whether or not and what type of territory (A, B, or C) you secured. Your reproductive success in that round will be calculated as follows:

<table>
<thead>
<tr>
<th>Territory Type</th>
<th>Energy Remaining</th>
<th># of Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>31–40</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>21–30</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>11–20</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>6–10</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1–5</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>31–40</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>21–30</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>11–20</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>6–10</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>31–40</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>21–30</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>11–20</td>
<td>1</td>
</tr>
</tbody>
</table>

After all reproduction has been calculated for every individual in the class, a new round of reproduction (a new breeding season) will begin and you will be given a new card with a number on it.

After three rounds, average energy remaining and average reproductive success for individuals in this population will be calculated.

Note: As indicated above, you may not, at any point, reveal to any other player how much energy you have remaining. You may, however, do whatever you wish with respect to facial expression, body posture, etc. to try to fool or bluff your opponent.

**Rules for Challenging and Energy Expenditure—Version II**

In this version of the game, the energy you have available for the breeding season will be represented by pennies contained within a paper bag. *You may not visually show the pennies in your bag to any other player at any time!*

Rules for challenging are similar to the previous version: if you approach a territory that already has an occupant, and you want the territory, and the occupant wants to retain control of the territory (as indicated by his/her continued presence at the territory), you must challenge the occupant. To do this, take one penny from your bag and place it on the table in front of you.

If you are an occupant and are so challenged, you must meet the challenge by placing one of your own pennies on the table in front of you. The fight continues with each player sequentially placing one of their pennies on the table in front of them.
Obviously, a player cannot place more pennies on the table than s/he already has in their possession!

If you decide that you do not want to continue the fight, place the pennies you invested in the fight in a pocket or purse; they may not go back into your bag. Leave the territory and either opt out or look for another territory. The territory winner should also put their invested pennies in a pocket. They represent energy expended in the fight that cannot be recovered. Do not let other players see how many pennies you put in your pocket or how many pennies you have remaining in your bag! You now have a certain number of pennies remaining with which to defend the territory you now occupy (if you won the previous bout) or to challenge for occupancy of another territory (if you lost).

The game continues as in the previous version until all territories are occupied and all other players have opted out of trying to secure a territory. Report your energy remaining and whether and what kind of territory you secured to the instructor. Reproductive success will be calculated as above, and you will be given a new set of pennies for the next breeding season.

Note: as indicated above, you may not reveal the pennies remaining in your bag at any time nor should you reveal how many pennies you lost during particular bouts. This does not, however, predicate against you using the bag with pennies in it to signal your potential ability to fight. Thus, new rule: signaling must be mutual (i.e., signaling by one player must be met by signaling by the other).

Follow-up activities

Work together in small groups to discuss the following questions. Each student should participate fully in the discussion so they can produce written responses to each question independently, should your instructor so require.

1. Did honest signaling of RHP increase or decrease the reproductive success of individuals in the class? What data do you require to be able to answer this question? How would you compare data from Version I with those from Version II? (Your instructor will provide information on appropriate statistical tests to use.)

2. Within the confines of the basic rules, what strategies did you each use in trying to “win” this game? Do you think that the different types of strategies affected the outcome of territorial bouts? Did you adjust your strategy during the game as you learned more about how it works?

3. Compare features of this game to models presented in one of more of the following papers: Maynard Smith and Parker 1976, Parker and Rubenstein 1981, the introductory section of Payne 1998, Taylor and Elwood 2003. What assumptions were made during this game that do not necessarily hold in other models of aggressive behavior? In what ways was this game similar to these other models?

4. Using the laboratory write-up as a template, modify this exercise to incorporate a change in one or more of the assumptions you identified in #3 (above). For example, you may recognize that there was only one strategy (non-signaling or signaling) played in each round, whereas in many models of aggressive behavior both strategies would compete against each other directly. Thus, you could re-write the rules of the game to include the possibility of signalers competing against non-signalers for access.
to territories. Can you incorporate changes in other assumptions into the rules of this game?

**Literature cited**


Exercise 18: Food & Fitness: A “First-Person” Simulation of Dragonfly Mating Ecology

Michael R. Maxwell and Paul V. Switzer

Goals
1. To generate and test predictions about the reproductive consequences of behavioral strategies
2. To explore the effects of certain traits on reproductive success
3. To learn about foraging behavior, reproductive physiology, mate choice, and life history trade-offs
4. To learn applications of basic probability

Background
This exercise is a “first person” simulation of animal behavior, in which you are to assume the role of a female dragonfly. As a dragonfly, you must juggle the tasks of avoiding starvation and predation, while ensuring insemination by the highest quality males possible. Your “score” (i.e., fitness), is calculated in terms of the quantity and quality of eggs that you lay. The simulation proceeds in discrete turns, and can be run entirely by a single student, or by a class with a moderator.

This simulation places animal behavior in a life history framework, where you decide between devoting energy to survival (i.e., somatic effort) and to the creation and fertilization of gametes (i.e., reproductive effort). As a female dragonfly, you make decisions about when and where to forage, the conversion of food to eggs, and criteria for mate acceptance. You also have a special trait, such as enhanced predator avoidance or perfect mate assessment, in your quest for bigger bellies and better babies. One of your goals is to see how these traits affect fitness.

The female dragonfly is a suitable organism for this simulation because, in many species, her foraging and reproductive behaviors are fairly separate (Corbet 1999). She spends her immature stages in the water, devouring other invertebrates and even some small vertebrates such as tadpoles and fish. Crawling from the water, she emerges as an elegant adult, taking to the air to feed and breed. She is extremely adept at catching flying insects with her spiny legs, eating her prey on the wing or at perch. Although she often forages and rests away from the water, she must eventually return to water to lay eggs. Oviposition sites, such as streams and ponds, are typically abuzz with males. Depending on the species, males defend egg-laying territories, or may simply intercept the female as she approaches the water. In dragonflies and other odonates, the female shows evidence of mate choice, selecting males based on territory quality or wing pigmentation (e.g., Siva-Jothy 1999, Corbet 1999). In some species of dragonflies, last-male sperm advantage is evident, where the last male to inseminate fertilizes the great majority of the female’s eggs (e.g., McVey and Smittle 1984). After insemination, she lays her eggs directly in the water or on aquatic plants. Her eggs now fertilized and deposited, the female returns to foraging. She can store unused sperm in special organs, and may use these sperm later to fertilize more eggs. As a reproductive adult, the female dragonfly typically lives for a matter of weeks (Corbet 1999).
Picture, then, yourself as a virgin female dragonfly. Your universe is a stretch of riparian forest in springtime. Darting along the riverbank are males—potential mates on patrol. Set back from the water are vibrant patches of flowers. Prey is abundant among the floral mosaic. Unfortunately, your predators, birds and carnivorous arthropods, are thick here as well. Farther back is a gallery of yew and willow—trees that may offer some haven at the cost of food.

**Hypothesis and Prediction Formulation**

Your goal is to gain as many “Fitness” points as possible. You get points by laying eggs, and you get more points by laying eggs fertilized by a high-quality male. Each student generates a Fitness score, so the class as a whole generates a “population” of Fitness scores. To guide the collection and analysis of this data set, your instructor is to choose one of two basic approaches to running this simulation: “Freeform” or “Fixed strategy.” The “Freeform” approach is rather exploratory, where all behavioral options are available at every turn. Before conducting this simulation, consider possible strategies that you can follow, as a dragonfly, in order to get the most fitness points. For example, there are two feeding areas, the flowers with higher prey abundance and predation risk, and the forest with lower prey abundance and predation risk. Which area do you expect to visit more? Why? Once you feed and create eggs, you “cash in” your eggs by getting them fertilized. Males, however, differ in quality, and a male’s quality determines the relative fitness value of your eggs. Is it better to hold out for a mate of highest quality, or to mate with the first male encountered? What reproductive consequences do you predict for either mating strategy? Your instructor may require you to write down your initial strategies and predictions. See whether you stick to your strategies, and note the circumstances that bring about any changes.

The “Fixed strategy” approach is a more rigorous design, where behavioral strategies are directly tested by conducting the simulation. This approach lends itself to deriving testable predictions from animal behavior theory. Your instructor may divide the class into groups, with each group following an explicit strategy. Three topic areas are suggested below, which reflect the main themes of this simulation.

1. **Foraging.** The flowers are “high food, high risk,” while the forest is “low food, low risk.” Thus, a “risky” strategy of “Always forage in the flowers” can be compared to a “cautious” strategy of “Always forage in the forest.” Rather than simply pitting two strategies against each other and running the simulation, form predictions about the fitness outcomes of each strategy. What factors might influence each strategy’s performance?

2. **Life history.** Ingested food must be converted into eggs. The conflict between somatic and reproductive effort can be brought into focus by positing food conversion strategies such as “Convert 90% of food into eggs” and “Convert 10% of food into eggs.” As above, formulate predictions about the relative outcomes of each strategy.

3. **Mate choice.** Sperm are needed for the eggs, yet not all sperm are created equal. A “Random” mating strategy (i.e., mate with every encountered male) can be compared to
one or more “Choosy” strategies, such as “Only mate with high quality males” or “Only mate with males of higher quality than stored sperm.” As above, generate predictions about the relative performances of each strategy. As you design your dragonfly, you may choose one of four traits: efficient foraging, antipredation ability, efficient egg production, and perfect mate assessment. Consider the different merits of each trait. If you are participating in a “Freeform” simulation, which trait do you think will be the most effective in maximizing your fitness? If you are participating in a “Fixed strategy” simulation, then your instructor may assign a trait to you in order to standardize possible effects of this variable. As you conduct the simulation, be mindful of situations when your trait directly affects your fitness.

**Methods**

**Simulation overview**

This simulation proceeds in turns. At the beginning of each turn, you are in your home refuge. In the refuge, you are fairly safe from predation. Your body’s metabolism, however, is always running, so you must venture out to feed and meet mates. At the start of the turn, you make three decisions: 1) Behavior (forage or reproduce?), 2) Egg Conversion Rate (how much energy do you devote to egg creation?), and 3) Mate Quality Threshold (how selective are you about your mates?). You, your classmates, or the moderator then determine what happens to you given the decisions you’ve made: Are you eaten by a predator? Do you find food? Do you mate and lay eggs? The answers to these questions affect state variables that describe your physiological status for the next turn (i.e., food, eggs, stored sperm).

You gain Fitness points when you lay fertilized eggs. The points earned by each egg are determined by the quality of the fertilizing male (ranked 1–5, where 1 = lowest and 5 = highest). For example, a fertilized egg of quality 5 is worth five times more than a fertilized egg of quality 1. Similarly, laying five quality 1 eggs is equivalent to laying one quality 5 egg. Your total Fitness is found by summing the values of the eggs that you lay every turn.

A score sheet (Data Sheet) is provided to aid in running the simulation. We provide suggested starting state variable values in Turn 1. These and other default values are given as guides, based upon our testing of the simulation. We do not claim that these are the ideal values, and encourage instructors and students to experiment with the numbers as they see fit.

**Getting started: becoming a female dragonfly**

Designing your life as a female dragonfly involves three steps: understanding the state variables, understanding the behavioral decisions, and selecting a special trait.

**State variables.** A set of state variables comprises the “engine” of your dragonfly. These determine how much energy you have (Food), how many eggs you may lay (Eggs), and the quality of your stored sperm (Sperm Quality). These are dynamic variables, meaning that they can change from one turn to the next.
1. **Food.** For simplicity, you start your life with 10 Food units. If you go to zero Food, you die from starvation.

2. **Eggs.** You start life with zero Eggs. During each turn, you convert food into eggs based on your stated Egg Conversion Rate (described below).

3. **Sperm Quality (1 to 5).** You begin life as a virgin adult, but store sperm after insemination. Sperm Quality equals the quality of most recent mate, ranked 1 to 5. For simplicity, your sperm supply is sufficient to fertilize all eggs that you may have, and does not deplete from one turn to the next.

### Decisions

You have an engine, but now need to control it. **At the beginning of each turn,** you make three decisions concerning feeding and reproduction (Behavior), egg production (Egg Conversion Rate), and mate choice (Mate Quality Threshold). Note that one or more of these decisions may be pre-determined in a “Fixed strategy” simulation.

1. **Behavior.** You have four options:
   a. Rest. Spend the turn in your refuge. Here, you burn some metabolic energy, are virtually free from predation risk, but will not find food.
   b. Forage in the forest. Spend the turn foraging in the forest. You increase your metabolic cost, but may find food with some predation risk.
   c. Forage in the flowers. Spend the turn foraging among the flowers. Your metabolic cost is the same as foraging in the forest, but food density is higher at the price of higher predation risk.
   d. Mate and Lay Eggs. Spend the turn visiting the riverbank to possibly mate and lay a stated number of eggs. You can specify that you will lay an exact number of eggs, or simply “All” of the eggs in your tract. You will encounter one male at the riverbank. If he meets your Mate Quality Threshold, then mating occurs, and you use his sperm to fertilize your eggs. If he is below your threshold, then no mating occurs, and you then use stored sperm (if any) to fertilize your eggs. Your Fitness for the turn is the number of laid fertilized eggs multiplied by the Quality of the sperm. If you did not have any stored sperm and did not mate, then you fail to lay eggs during the turn.

2. **Egg Conversion Rate.** This determines what percentage of your Food is converted into Eggs during the turn (i.e., 0 to 100%). Animals are not perfect machines, so conversion into eggs is not 100% efficient—only 75% of the allocated Food converts into eggs. For example, suppose you have 10 Food units and decide to convert 40% into Eggs. You actually create 3 eggs (i.e., $10 \times 0.4 \times 0.75 = 3$ eggs), while your Food drops by 4 units (i.e., $10 \times 0.4 = 4$ food units).

3. **Mate Quality Threshold.** This determines your minimum acceptable mate quality (i.e., 1 to 5). You are sometimes incorrect in your assessment of a male’s quality, however. You misidentify a male’s quality 33% of the time. Assessment errors are equally likely to over- or under-estimate the male’s true quality.

### Trait

You can now control your engine, so let’s give it a special feature. To reflect phenotypic variation among females, you choose one special trait for your dragonfly. This trait remains the same throughout the entire simulation.

1. Efficient forager: twice as likely to find food in a given habitat.
2. Reduce predation risk: half as likely to be captured by a predator in a given habitat.
3. Efficient egg conversion: Food is converted into Eggs with 100% efficiency.
4. Mate assessment: always accurately identify male quality.
**Running the simulation**

So, you have designed your super-charged dragonfly. Now, let’s see how she performs. The survival and reproductive outcomes for your dragonfly in any given turn depend on an interaction between your state variables, decisions, and selected trait. Below, we outline how to run a turn of the simulation. You will need three dice, a coin, and your Simulation Sheet in hand.

**Step 1: Make the three decisions.** Select which Behavior you will perform, and select values for Egg Conversion Rate and Mate Quality Threshold.

**Step 2: Find the outcomes for your Behavior**

*Behavior: Rest.*

1. Metabolism. For resting, you incur a minimal metabolic cost of 1 Food unit. Make a note of this on your Simulation Sheet.
2. Predation? To determine whether you are eaten by a predator when at rest, roll three dice. If you roll three 1s (probability = 0.0046), then you are eaten. If you are eaten and have the “Reduce predation risk” trait, then flip the coin. If it lands as Heads, then you manage to escape death at the last second.
3. Food found. Does not apply for this behavior.
4. Eggs created. Multiply your Food state variable at the start of the turn by your Egg Conversion Rate. Multiply this product by 0.75, or by 1.00 if you have the “Efficient egg conversion” trait. Round the final answer to the nearest integer and write it on your sheet.
5. Mate? Does not apply for this behavior.
6. Eggs laid. Does not apply for this behavior.

*Behavior: Forage in the forest.*

1. Metabolism. Metabolic cost is 2 Food units.
2. Predation? Roll two dice. If you roll two 1s (probability = 0.028), then you are eaten. If you are eaten and have the “Reduce predation risk” trait, then flip the coin. If it lands as Heads, then you manage to escape death.
3. Food found. Roll one die. If you roll a 1, 2, or 3 (probability = 0.5), then you find food. If you have the “Efficient forager” Trait, then you automatically find food. If you find food, roll one die and add 2 to the result. This sum is the amount of Food units that you eat.
4. Eggs created. First, add your Food state variable at the start of the turn to any “Food found.” Then, multiply this sum by your Egg Conversion Rate. Multiply this product by 0.75, or by 1.00 if you have the “Efficient egg conversion” trait. Round the final answer to the nearest integer and write it on your sheet.
5. Mate? Does not apply for this behavior.
6. Eggs laid. Does not apply for this behavior.

*Behavior: Forage in the flowers.*

1. Metabolism. Metabolic cost is 2 Food units.
2. Predation? Roll one die. If you roll one 1 (probability = 0.167), then you are eaten. If you are eaten and have the “Reduce predation risk” trait, then flip the coin. If it lands as Heads, then you manage to escape death.
3. Food found. You automatically find food in the flowers, regardless of whether you are an “Efficient forager.” Roll one die and add 2 to the result. This sum is the amount of Food units that you eat.

4. Eggs created. Add your Food state variable at the start of the turn to the “Food found.” Multiply this sum by your Egg Conversion Rate. Multiply this product by 0.75, or by 1.00 if you have the “Efficient egg conversion” trait. Round the final answer to the nearest integer and write it on your sheet.

5. Mate? Does not apply for this behavior.

6. Eggs laid. Does not apply for this behavior.

**Behavior: Mate and Lay Eggs.** You must write in how many eggs you want to lay on the space provided on the Simulation Sheet.

1. Metabolism. Metabolic cost is 1 Food unit.

2. Predation? Roll two dice. If you roll two 1s (probability = 0.028), then you are eaten. If you are eaten and have the “Reduce predation risk” trait, then flip the coin. If it lands as Heads, then you manage to escape death.

3. Food found. Does not apply for this behavior.

4. Eggs created. Multiply your Food state variable at the start of the turn by your Egg Conversion Rate. Multiply this product by 0.75, or by 1.00 if you have the “Efficient egg conversion” trait. Round the final answer to the nearest integer and write it on your sheet.

5. Mate? To determine the quality of the encountered male, roll two dice. Add the two rolled numbers together. Determine male quality by the following table:

<table>
<thead>
<tr>
<th>Sum</th>
<th>Male quality (True)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
<td>0.028</td>
</tr>
<tr>
<td>3-5</td>
<td>2</td>
<td>0.250</td>
</tr>
<tr>
<td>6-8</td>
<td>3</td>
<td>0.444</td>
</tr>
<tr>
<td>9-11</td>
<td>4</td>
<td>0.250</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Through your roll, you have determined the male’s True quality. Now you will determine your actual perception of the male by the following algorithm:

- If you have the “Mate assessment” trait, then Perceived quality = True quality.
- If you do not have the “Mate assessment trait, then roll one die.
- If you roll 1-4, then Perceived quality = True quality.
- If you roll 5 or 6, then flip a coin. If Heads, then Perceived quality = True quality + 1. If Tails, then Perceived quality = True quality - 1.

If the resultant Perceived quality is equal to or greater than your Mate Quality Threshold, then you mate with the male, and his sperm completely replaces any previously stored sperm. If the Perceived quality is lower than your threshold, then you do not mate.

6. Eggs laid. If you mated with a male during this turn, or have stored sperm (i.e., there is a numerical entry for your Sperm Quality state variable), then you may lay fertilized eggs. You lay as many eggs as you stated, provided that the sum of your Eggs state variable and the “Eggs created” is equal to this number. If this sum is less, then you only lay the sum. If the sum is more, then you lay the stated number with the extra eggs remaining in your tract.
Step 3: Calculate Fitness for the turn. If you laid fertilized eggs, multiply the number of eggs that you laid during the turn by either a) the True quality of the male with whom you mated during the turn, or b) the quality of your stored sperm (i.e., Sperm Quality state variable at the beginning of this turn). Enter this product into the “Fitness” box for the turn.

Step 4: Determine new state variables and decisions for next turn. If you did not succumb to predation, then you live on for another turn. Your state variables at the start of the new turn are determined by the following rules.

\[
\text{Food} = (\text{Food at start of last turn}) \\
+ (\text{Food found during last turn}) \\
- [(\text{Food at start}) + (\text{Food found})] \times [\text{Egg Conversion Rate}] \\
- (\text{Metabolism for Behavior during last turn})
\]

\[
\text{Eggs} = (\text{Eggs at start of last turn}) \\
+ (\text{Eggs created during last turn}) \\
- (\text{Eggs laid during last turn})
\]

\[
\text{Sperm Quality} = \begin{cases} 
\text{Sperm Quality at start of last turn (if no mating during last turn)} \\
\text{or} \\
\text{True quality of male mated with during last turn}
\end{cases}
\]

Before you go on, check your new Food. If it is zero (or less), you have starved to death. Better luck in your next run, but at least your dragonfly is in a better place. If you still have Food in your gut, then you can now make new decisions, keeping in mind your new state variables. With your new decisions, you can then determine the outcomes as described above.

Stopping the simulation

The simulation itself is rather open-ended, with no fixed stopping point. Your instructor may specify a certain number of turns, at which time all dragonflies might sum up their total Fitness. Dragonfly deaths most likely will occur. How to handle these is up to you and your instructor: you might sit out for the rest of the simulation, or you may be reincarnated as a new dragonfly with default values at the start of the next turn.

Questions and/or Report Instructions

1. If you participated in a “Fixed strategy” simulation, did the comparative Fitness scores of the strategies match your predictions? If not, what were the likely factors that caused these results? How would the simulation’s rules or parameter values have to change to have your initial predictions confirmed?

2. If you participated in a “Freeform” simulation, comment on any overall behavioral strategies that you outlined before running the simulation, such as “Always forage in the flowers” or “Only mate with highest quality males.” Did you follow these strategies? If not, what brought about the changes?

3. Compare your dragonfly’s performance with those that had other special traits. Which did better? How well were your initial predictions about the effects of the traits borne out? What factors or outcomes were responsible for supporting, or not supporting, your predictions?
4. If you participated in a “Freeform” simulation, how were your turn-by-turn decisions affected by your chosen trait? How did your decisions change based on your experience? Given that your chosen trait was fixed throughout the simulation, how might your trait and experience reflect the roles of genetics and learning in animal behavior?

5. Reflect upon your turn-by-turn decisions (and those of your classmates) regarding behavior, egg conversion, or mate quality threshold. Do any patterns emerge with respect to foraging behavior or mate choice? If you’ve studied optimal foraging theory and models of mate choice in your class, discuss how well your results fit in.

**Acknowledgments**

The design of this exercise was inspired by dynamic state variable modeling as presented in Mangel and Clark (1988). We thank students at Eastern Illinois University (USA) and National University (California, USA) for bravely conducting simulations of previous versions of this exercise.

**Literature Cited**


