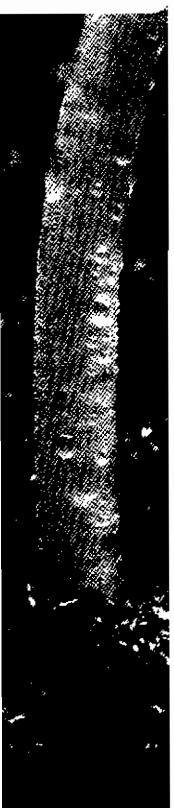


Classroom Mark-Recapture with Crickets

ANDREW R. WHITELEY
JENNIFER WOOLF
KATHLEEN KENNEOY
DAVID OBERBILLIG
CAROL BREWER

How many deer live in a particular county? How many fish live in the creek that runs near a school? And how have these numbers changed over time in the last 10 years? These are the questions of population ecologists. Population ecology is the study of changes in the abundance of organisms over time and space (Akcakaya et al., 1999). Temporal and spatial trends of animal abundance are commonly used to prioritize conservation and management efforts for various animals. For example, these trends are used to help determine the number of hunting permits that will be issued in a given year. Due to its central role in ecology, many high school and undergraduate biology courses include lessons on population ecology theory.

Curriculum pieces on population ecology theory often include investigations on methods used to estimate animal abundance. One commonly used technique to estimate the size of natural populations is single mark-recapture using the Lincoln-Petersen model (Smith & Smith, 2001). In this method, animals are captured, given an identifying mark such as a paint spot or a tag with a number, and then released back to their habitat. At a later date, traps are set again in the same places. The ratio of marked to unmarked animals during the second capture event can be used to estimate the size of the population. This method provides a simple means to estimate the population size of animals. The basic form of the Lincoln-Petersen model is mathematically straightforward and appropriate for teaching about mark-recapture methodology. The basic model also provides an ideal mechanism for integrating science and mathematics.

Various strategies have been used to teach mark-recapture in high school and undergraduate classrooms. A common teaching strategy uses dried beans or plastic beads as model animals (e.g., Budnitz, 1998). In this strategy, a subsample of beans or beads is taken out of a container, marked, and returned to the container. In a subsequent "recapture" event, another subsample of beads is collected and the proportion of marked beads relative to the number of unmarked beads is used to estimate the total "population size" of beads. While this activity is relatively simple to perform in the classroom, we have found that it often does not work well because too many beads are in the containers to start with and students usually do not mark a large enough proportion of the beads during the mark and recapture trials to obtain accurate population estimates. This inaccuracy can erode student interest and enthusiasm. It is possible to increase the accuracy of this method by making sure a larger proportion of beads is initially marked, however, this activity can then become tedious for students. More importantly, this type of exercise does not present mark-recapture as it is used in practice and it does not engage students by having them handle and learn about living organisms.

Another teaching strategy involves the use of live animals, either in schoolyards (Anonymous, 2002; Schimmelpfennig & Schneider, 2005), or in a more natural field setting (Dussart, 1991; Rollinson, 2004). Working with live organisms provides an opportunity for students to learn about the natural history and biology of the animals. This is an excellent option if large populations of easily captured organisms are available close to a school, such as various types of non-biting insects. We believe that working with live animals is inherently more interesting to the students. Moreover, students get out of the classroom and into nature. However field-based investigations present three challenges that detract from how well they can be used to teach about mark-recapture. First, working in a field setting requires significant planning and some uncertainty about the likelihood of successfully capturing and then recapturing enough animals. Second, population estimates can be problematic if the assumptions of mark-recapture models are not satisfied. Finally, the true number of animals is unknown in a field experiment and therefore estimates obtained cannot be compared to true values, which is useful for instructional purposes.

An intermediate approach that shares the advantages but lacks the disadvantages of the two teaching strategies just described is to use closed populations of live animals to perform a classroom based mark-recapture investigation. This approach shares the advantage of the bead exercise in that students conduct the investigation in the classroom so there is no uncertainty about finding enough animals. It shares the advantage of field-based investigations in that

ANDREW R. WHITELEY is International Polar Year Postdoctoral Fellow, University of Alaska Southeast, Juneau, AK 99801; e-mail: andrew.whiteley@uas.alaska.edu. JENNIFER WOOLF is a Ph.D. Student, College of Forestry and Conservation, University of Montana, Missoula, MT 59812; e-mail: jenniferwoolf@hotmail.com. KATHLEEN KENNEDY (kkennedy@mcps.k12.mt.us) is a biology teacher at Big Sky High School, Missoula, MT 59804. DAVID OBERBILLIG (doberbillig@mcps.k12.mt.us) is a biology teacher at Hellgate High School, Missoula, MT 59801. CAROL BREWER is Professor, Division of Biological Sciences, University of Montana, Missoula, MT 59812; e-mail: carol.brewer@umontana.edu.

students work with and must handle live organisms, which also provides an opportunity for students to learn about the chosen animal's biology. Despite the advantages of this intermediate approach to teaching about mark-recapture, we are aware of few investigations available to teachers (however for an example that uses fish, see Haag & Tonn, 1998).

In the investigation described here, we use crickets living in a 10-gallon aquarium habitat to teach mark-recapture techniques using live animals in a classroom setting. We chose crickets for this activity because they are commonly available at most pet stores and they are inexpensive. The motivation for developing this investigation was to provide a more integrated picture of how biologists use sampling and mathematics to estimate population size. This investigation is designed to complement other population ecology curricula in general high school biology courses, but could also be used in AP biology, freshman biology, and ecology courses.

Figure 1. Glossary

SAMPLING TERMINOLOGY

Closed population: a population where no births or deaths occur and individuals do not enter (immigrate) or leave (emigrate) during the time of study.

Confidence interval (CI): The range in which you expect to find 95% of all estimates.

Lincoln-Petersen model: a specific mark-recapture technique that requires two sessions during which animals are captured. This is a basic technique that forms the basis for more complicated population estimation methods. The equation for the model is:

$$\frac{n_1}{\hat{N}} = \frac{m_2}{n_2}$$

where: n_1 is the number animals marked and released during the first session; n_2 is the number of animals captured during the second session; m_2 is the number of animals captured during the second session that are recaptures from the first session; and $\hat{N}$ is the estimate of population size.

Mark-recapture techniques: a set of techniques used to estimate the population size of animals. All of the techniques involve marking animals the first time they are captured and then releasing them. Animals are recaptured a second time and the proportion of marked to unmarked animals is used to estimate the population size.

Standard error (SE): an estimate of how much variation there is about the mean population estimate.

Subsample: using a smaller group collected from within a larger population of "objects."

Cricket Anatomy: (Borror et al., 1992)

Insects of three primary body regions (the head, thorax, and abdomen):

Head: the anterior body region, where you find the eyes, antennae, and mouthparts.

Thorax: the body region behind the head, with the legs and wings.

Abdomen: the posterior region of the body (no legs or wings).

Ovipositor: the egg laying apparatus; the external genitalia of the female.

Cercus (plural cerci): one of a pair of appendages at the posterior end of the abdomen.

Objectives of This Activity

The general goal of this investigation is to complement instruction on population ecology and to teach mark-recapture theory and techniques that are used by population biologists to understand the distribution of animals in space and time. More specifically, students learn:

- mark-recapture techniques to estimate population size of naturally occurring organisms
- how to calculate a population estimate by algebraic manipulation of simple ratios and solving equations for one unknown with data they collect
- about the natural history and biology of a common insect
- to consider how wildlife biologists estimate population sizes and about popular press stories that feature abundance estimates of wild animal populations.

This investigation promotes science as inquiry and helps students develop skills in asking questions, collecting and interpreting data, and communicating the results with their peers. It maps easily onto the *National Science Education Standards* (NRC, 1996). This guided investigation can lead to more open-ended inquiries (Content Standard A). Moreover, it emphasizes concepts related to population growth and natural resources (Content Standard F). Finally, students refine their ability to use models and equations to make estimates and predictions (Content Standard G).

Table 1. Materials for cricket investigation.

Number needed for six groups is in parentheses.

MATERIALS NEEDED FOR INVESTIGATION

- 10-20 gallon aquarium (1)
- "Pet store" crickets (~50)
- Cardboard "traps" ("cricket castles"). We used egg cartons and cardboard packing material from an electronic device (~10)
- "Painters" acrylic non-toxic paint pens (Hunt Inc.; number of colors depends on number of participating classes)
- Chopped apple (provides food and water for crickets)
- Large (32 oz) plastic containers (6)

Counting Crickets

Materials

Table 1 provides a list of the materials needed for this activity. We recommend using the same set of approximately 50 crickets for multiple classes. Each class can mark a different body part and each class should use a different colored paint pen. Using the same set of crickets for multiple classes minimizes setup time for the instructor and forces the students to be careful with how and where they mark the crickets.

Investigation

This investigation can be completed within a one to one-and-a-half hour class period. There are two short periods of time for direct instruction and two periods where students first capture, handle, and mark crickets and then later perform calculations for population size estimates. A more detailed version of this investigation and a PowerPoint file with masters for overhead transparencies are available online at: http://www.bioed.org/ecos/inquiries/inquiry_crickets.pdf.

At the beginning of each class session, students receive a handout that briefly explains the investigation and contains a data sheet for them to complete (Figure 2). Students work in groups and each group receives one data sheet. We recommend group sizes of three students.

The first lecture was designed to build on previous population ecology lessons and activities. In this lecture, the focus is on why it is important to estimate the population size of naturally occurring animals. The following concepts are addressed:

- the importance of estimating population sizes of naturally occurring animals
- the basic ideas behind mark-recapture techniques
- the importance of understanding the biology and natural history of the animals we study
- basic insect anatomy; specifically how to mark crickets; safety and ethical issues with working with live animals
- general logistics.

Figure 2. Description of Investigation and Data Sheet.

CRICKET MARK-RECAPTURE INVESTIGATION

Name: Period: Date:

For this investigation, we will estimate the population size of crickets in an aquarium. You will work in teams of three to catch, mark, release, and recapture crickets. Each team will take a plastic container to the aquarium and capture crickets by scooping out a set of crickets (scoop only once!). This is your first sample. Take the crickets in your container to your desk and mark all of these crickets where your teacher indicates, using a paint pen. Fill in the number of crickets caught during your first sample for n_1 in the data table.

Once everyone has caught and marked crickets, each team will return the marked crickets to the same aquarium. We will wait 15 minutes. Then each team will take a second sample of crickets. Again, take the container of crickets to your desk and record the total number of crickets caught. Also record the total number of crickets with marks. The total number of crickets you caught the second time is n_2. The number of crickets you caught the second time with marks is m_2. Fill these numbers in below. Also record whether each cricket is male or female and whether or not it has wings in the table at the bottom of the page.

Your group's totals:

n_1 = ____________ n_2 = ____________ m_2 = ____________

As a class, we will pool our cricket samples to estimate the abundance of crickets in the aquarium.

Class totals:

n = ____________ n_2 = ____________ m_2 = ____________

Data needed for EACH cricket. Fill in the data on sex and wings in this table:

Cricket #	Sex (M/F)	Wings (Y/N)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Fill in the TOTAL number of males and females with and without wings in this table:

Winged/wingless	Males	Females
Wings		
Wingless		
Total		

There are minimal safety issues associated with this investigation but students should wash their hands after handling the crickets. More detailed information on these topics is available at http://www.bioed.org/ecos/inquiries/inquiry_crickets.pdf. We wait to explain details of the Lincoln-Petersen model until after the first capture session.

The first step in the investigation is for each group to collect crickets from the aquarium at the back of the room. One student from each group removes one "cricket castle," which is a small portion of an egg carton (Figure 3), and gently shakes the crickets from the egg carton into a plastic container. Each group returns to its table with its crickets. Five to six crickets per group works well but this will vary according to the number of crickets in a given "castle." It is important to provide enough pieces of egg carton so that each group can use one, and it is also helpful for the instructor to supervise the capture process so that groups overturn only one piece of egg carton. We observed a tendency for the students to overturn many of the pieces of egg carton and to disturb many of the crickets if we left them unsupervised.

At their table, the groups use a paint pen to mark the crickets. One student holds a cricket while another dabs the specified body part with paint, and a third records the number of crickets marked (this is n_1 in the equation described below and in Figure 2). Each class uses a different colored paint pen and marks either part of the thorax or one of the legs of the crickets. Students also record data (using the supplied data sheet) on whether or not the crickets have wings and the gender of each cricket. After all of the groups obtain crickets, the first groups are allowed to gently return crickets to the aquarium.

Once the crickets are back in the aquarium, the theory behind mark-recapture using the Lincoln-Petersen method is presented during a second 15-minute lecture. This lecture focuses on the variables in the Lincoln-Petersen estimate of abundance, the ratio used to calculate $\hat{N}$ (the estimate of population size), and the assumptions of the model. This break in activity allows the crickets to settle back into their "traps." We found that 15 minutes provided ample time for this second lecture and for crickets to redistribute themselves in the aquarium.

The equation for the Lincoln-Petersen model is:

$$\frac{n_1}{\hat{N}} = \frac{m_2}{n_2} \quad \text{(Equation 1)}$$

where n_1 is the number of animals marked and released during the first session, n_2 is the number of animals captured during the second session, m_2 is the number of animals captured during the second session that are recaptures and were marked during the first session, and $\hat{N}$ is the estimate of population size. This equation can be algebraically manipulated to solve for $\hat{N}$, such that

$$\hat{N} = \frac{n_1 n_2}{m_2} \quad \text{(Equation 2).}$$

This model has several important assumptions that are important to discuss with the class (Table 2).

In addition to calculating $\hat{N}$, an optional extension for advanced students is to calculate the standard error of the estimare of population size using the following equation:

$$S.E. = \hat{N}\sqrt{\frac{(\hat{N}-n_1)(\hat{N}-n_2)}{n_1 n_2(\hat{N}-1)}} \quad \text{(Equation 3).}$$

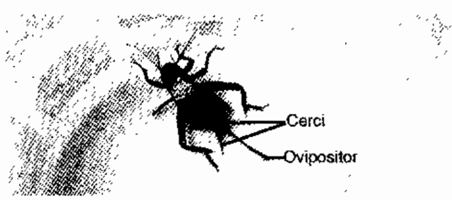
$\hat{N} \pm 2(S.E.)$ provides the 95% confidence interval about $\hat{N}$ (Smith & Smith, 2001).

Finally, the factors that might lead to differences between the students' estimate of population size and the true population size are discussed. Equation 2 and the model assumptions (Table 2) are used as a guide for this discussion. Students are asked to think about this equation and the effects of violations of the assumptions of the model on the different variables in this equation. For example, if the crickets lost their marks between the capture and the recapture sessions, this would lead to an upwardly biased estimate of the population size because m_2 will be biased low and since this is in the denominator of Equation 2, $\hat{N}$ will increase.

The recapture event follows this second lecture. Groups repeat the same process of capturing crickets described above. Students record m_2 (the number of marked crickets captured during this session) and n_2 (the total number of crickets their group recaptured). Each group reports n_1, n_2, and m_2 in a table made by the instructor on the board. The sum of each variable is used as the class total to estimate $\hat{N}$ for the entire class (Figures 2 & 5).

Figure 3. Crickets on a cricket "castle."
The castle is a cardboard insert to an electronic appliance. Notice the paint on some of the crickets, particularly the white on the back and purple on the leg of the cricket at the top of the photo (photo by A. Whiteley).

Figure 4. Cricket in a yogurt container.
The thorax of this cricket has been painted by students with a white paint pen. The cerci extend from the back of the abdomen. The ovipositor is at the very tip of the abdomen (photo by A. Whiteley).



After students work through the calculations of $\hat{N}$, a general discussion follows about how close the estimate is to the true value. We recommend an interactive discussion with questions that require students to consider the effects of violations of the model (Table 2) on the variables in Equation 2. Reasons why $\hat{N}$ might not be accurate are discussed, along with confidence intervals (optional), and potential violations of assumptions. Refer to the list of model assumptions (Table 2 and listed on an overhead in supplemental online material) to discuss each assumption and whether it may have been violated. For example, "cricket escapes" would violate the closed population assumption. Another possible source of bias could be related to the trapping method used in this investigation. $\hat{N}$ might be biased low because stressed crickets might crawl directly back into the castles to seek cover after the crickets are placed back into the aquarium. Thus, m_2 might be biased high, in turn causing $\hat{N}$ to be biased low.

Students are also instructed to think about the data they collected on the number of males and females that have wings. These data are used to generate hypotheses. For the crickets we used in this investigation, females tended to be wingless while males had wings. One hypothesis is that females might not have wings because of the way they allocate their limited resources to reproduction versus growth. Because they use a lot of energy to make eggs, fewer energy-related resources may be devoted to growing wings during development. Males may need to allocate energy to the production of wings because they might disperse more than females, perhaps to find mates.

Table 2. Assumptions of the Lincoln-Petersen model.

ASSUMPTIONS OF LINCOLN-PETERSEN MODEL

1) Population is closed (no births, deaths, immigration, or emigration).
2) Marks are nol lost or overlooked by observer.
3) All animals equally likely to be captured in each sample (no differences among individuals in preference or avoidance of trap).
4) Trapping probabilities are equal during the two trapping periods.

A follow up exercise to increase student comprehension is provided in Figure 5. Students are given time to answer the questions after the class' population estimate is discussed.

Extensions

Genetics techniques are now often used to estimate the population size of animals. For example, biologists in Glacier National Park, Montana, use hair collected from special hair-snagging stations to estimate the number of bears in the park (for more details see: http://www.nrmsc.usgs.gov/research/glac_beardna.htm). Some of the techniques used to analyze genetic data use extensions of the Lincoln-Petersen model. Thus, after learning the basic theory and technique using the crickets, an additional lesson could further explore indirect genetic methods to estimate population size.

As another extension, students can design their own research investigation to use mark-recapture with naturally occurring populations of animals, perhaps as part of an independent project. This could be done in the schoolyard with insects using a similar technique described in this paper (e.g., pillbugs; Anonymous, 2002). If a pond is nearby, frogs can be marked by clipping toes. Guidelines for toe-clipping can be found at: www.asih.org/pubs/ASIH_HACC_Final.pdf. For a discussion of the ethical aspects of this technique, see Funk et al. (2005). If fish can be captured, individuals can be marked by clipping small portions of fins. Note that these more invasive techniques (e.g., clipping any body part of an animal) can only be performed with the consent of animal care committees and/or local fish and wildlife departments. We recommend contacting a local university or fish and wildlife department if students are interested in undertaking a project like this. There may be projects underway with opportunities for participation by volunteers.

Figure 5. Questions and problems to accompany investigation.

Answers are italicized.

QUESTIONS FOR CRICKET MARK-RECAPTURE INVESTIGATION

1) Use the class data to estimate the population size of crickets in the aquarium ($\hat{N}$). Show your work below and use this equation:

$$\hat{N} = \frac{n_1 n_2}{m_2}$$

If, for example, $n_1 = 22$, $n_2 = 22$, and $m_2 = 10$, then $\hat{N} = \frac{(22)(22)}{10} = 48.$

2) Which assumptions of the Lincoln-Petersen model might we have violated?

Potential assumptions that we violated are: populations are closed (no births, deaths, immigration or emigration), marks are not lost or overlooked, and all animals are equally likely to be captured during both capture events. It is possible that crickets were lost, marks were lost or overlooked, and individual crickets may have been more or less likely to be captured during either capture event.

3) How would the violations you mentioned effect your estimate of population size ($\hat{N}$)?

The effect of each potential violation on the estimate of population size can be determined using Equation 1. For example, $\hat{N}$ might be biased low because stressed crickets might crawl directly back into the castles to seek cover after the crickets are placed back into the aquarium. Thus, m_2 might be biased high, in turn causing $\hat{N}$ to be biased low.

4) If the class estimate was close to the actual number of crickets in the aquarium, does this guarantee that we didn't violate any assumptions of the Lincoln Petersen model? Why or why not?

No. If our estimate was close, this does not guarantee that we did not violate some of the assumptions of this model. Perhaps an assumption was violated but this violation did not have a large effect on the estimate of population size, or perhaps multiple violations cancelled each other out.

5) Of what value for society is estimating the size of naturally occurring populations?

There are many reasons for estimating the size of naturally occurring populations. As mentioned in the text, these estimates form the foundation of many ecological studies (e.g., for species interactions, we often need to have an estimate of how many of the interacting species are present in the environment) as well as for conservation and management priorities and actions. For example, estimates of population size are used to determine the number of hunting tags to issue in a given region.

Did This Investigation Provide a Successful Learning Experience?

This investigation was tested with high school sophomores in their second year of a biology series. We thought it was a substantial improvement over the bead exercise used in the past for two reasons:

1) It provided an estimate of population size that was closer to the true population size more often

2) It seemed to better capture student interest.

The classes' population estimates were close to the true value of 50 crickets in the aquarium and most estimates were within 10% of the true value; only one was off by 16% from the true value. In contrast, the bead investigation, as it is normally conducted, often yielded results that were highly inaccurate, causing students to doubt the efficacy of the technique and, as a consequence, diminished their interest. It is our opinion that the challenge of handling and marking live animals was a large part of the appeal of this exercise. Many students had to confront their fear of insects and most appeared to enjoy handling the crickets.

We also examined how well students performed on the assessment problems and questions (Figure 5). While we did not perform a rigorous analysis, we found that students performed well on these questions and we conclude that students gained an overall understanding of mark-recapture theory and technique. In general, students successfully manipulated equations, were able to think carefully about assumptions of the Lincoln-Petersen model, and gave thoughtful responses regarding the broader importance of estimating the size of natural populations. A smaller subset of the students had difficulties using and manipulating Equations 1 and 2 (we did not use Equation 3 for the standard error) and there was wide variation in answers related to assumptions of the model. We found that these concepts were important to revisit through additional problems, questions, and class discussion.

Conclusions

This population ecology investigation provides an inexpensive way to teach students, in a real world context, about a technique commonly used in field biology and ecology. This approach for teaching about mark-recapture methods provided highly accurate estimates of cricket abundance and appeared to capture student attention more than the typical bean or bead counting strategies for teaching the same material. This investigation is easily done in any classroom setting. Moreover, it has the advantages of allowing students to handle live insects without the drawback, uncertainty, and time necessary to teach mark-recapture in a field setting. Finally, through class discussion, we were able to make a link between this investigation and the practices wildlife biologists use to estimate population sizes. We also connected the investigation to popular press stories featuring abundance estimates of wild animal populations, thus increasing student understanding of conservation issues.

Acknowledgments

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