Marked Cards in the Pack: Using Playing Cards to Teach the Importance of Sample Size & Testing Assumptions in Capture–Recapture Estimations of Population Size

RECOMMENDED FOR AP Biology

MICHAEL CALVER, TIMOTHY BLAKE

ABSTRACT

Estimating population size is essential for many applications in population ecology, so capture–recapture techniques to do this are often taught in secondary school classrooms and introductory university units. However, few classroom simulations of capture–recapture consider the sensitivity of results to sampling intensity, the important concept that the population size calculated is an estimate with error attached, or the consequences of violating assumptions underpinning particular capture–recapture models. We describe a simple approach to teaching the Lincoln index method of capture–recapture using packs of playing cards. Students can trial different sampling intensities, calculate 95% confidence intervals for population estimates, and explore the consequences of violating specific assumptions.

Key Words: *mark—recapture; animal populations; Lincoln index; population estimates; confidence interval; assumptions.*

○ Introduction

Estimates of population size for animal species are fundamental to many key questions in ecology, including population trends in threatened species (Hunter et al., 2010), assessing the effectiveness of pest control programs (Marlow et al., 2015), sustainable harvest (Naves, 2018), and monitoring the spread of invasive species (Musseau et al., 2015). However, estimating population size poses special challenges for animal ecologists because of the mobility of most animal species. One approach to estimating animal populations is to use a capture–recapture technique in which a sample of animals is caught, tagged, and released to rejoin the population. The population is then resampled at a later date and the proportion of tagged animals in the new sample is used to estimate the overall population size (Borchers, 2012).

"In the simulation, the pack of cards represents the population of animals under study."

Ideally, students would study such techniques on an animal species in the field, but logistics and animal welfare issues mean that most teachers use a simulation. There is much ingenuity here, with examples including the use of taxis (Bishop & Bradley, 1972; Bishop, 1979), shopping carts (Gilhooley, 1985), model animals (Mudie & Brotherton, 1984; Masene, 2001; Goetze & Rodriguez, 2018), counters on a board (Calver et al., 1990), computer simulations (Steiner, 1983), and laboratory cultures of live animals (Whiteley et al., 2007). Most of these examples follow the basic steps of sampling, tagging, resampling, and using the proportion of tagged animals in the second sample (the recaptures) to estimate population size using the Lincoln index. However, not all these examples consider the sensitivity of results to sampling intensity or the vital concept that population size is estimated and that there is an error on the estimate. Others do not explain that the Lincoln index is valid only if explicit assumptions about the population under study hold (e.g., that all individuals in a population have an equal probability of being captured).

Here, we show the significance of sampling intensity, the errors in population estimates, and the consequences of violating assumptions in a simulation using playing cards. Sampling intensities can be varied, 95% confidence intervals for population estimates calculated, and assumptions violated to explore the consequences for population estimations.

○ Resources Required & Preclass Preparation

Prior to class, students should read any relevant sections of their textbooks that cover population estimation. Students will work in groups of two to four, with one standard pack of 52 playing cards (jokers removed) per group. Each group will need access to random number tables or to a random number generator. Each will also need either an internet connection, for access to online

calculators for binomial standard errors, or copies of the charts in Krebs (1999, p. 27) for estimating binomial standard errors – unless the instructor wishes students to calculate the latter themselves. The instructor should decide this before class, and provide students the instructions for either approach as part of the procedures described below.

○ Briefing in Class

Before beginning the exercise, students can be asked to

- suggest reasons why it is often helpful to estimate the size of animal populations;
- list logistic and ethical problems that might arise when estimating animal populations in the field;
- outline the steps involved in a capture–recapture study using the Lincoln index;
- list important assumptions that must be met for a population estimate based on the Lincoln index to be valid; and
- give their opinion on how accurate a population estimation based on the Lincoln index is likely to be.

Students often give good answers to the first two points, while the quality of discussion regarding the others is often related to whether or not they did the background reading. The last point on the accuracy of the population estimation usually generates disagreement, which can be used to focus on questions of sampling intensity, stating confidence intervals for population estimations, and awareness of assumptions underpinning the estimates. The simulation allows students to evaluate their opinions.

○ Procedure for Assessing Effects of Sampling Intensity

In the simulation, the pack of cards represents the population of animals under study. One-third of the groups work at a sampling intensity of 10 "traps," one-third at 20 "traps," and one-third at 40 "traps." The procedure for each group is as follows:

1. Shuffle the cards thoroughly.
2. Use random number tables to determine the number of traps that have "caught" an animal (e.g., if the sampling intensity is 10, take a random number between 0 and 10, if the sampling intensity is 40, take a random number between 0 and 40).

3. Deal out as many cards as were determined in step 2 and record each card (Table 1 can be used as a template). These are the "animals" caught in the first sample.
4. Return the cards to the deck and shuffle thoroughly.
5. Use random number tables to determine the number of traps that "catch" an animal on the second sampling occasion, assuming the same sampling intensity as in step 2.
6. Deal out as many cards as were determined in step 5 and record each card, noting any cards that are "recaptures" from the first sampling occasion.
7. Estimate population size using the Lincoln index:

$$\hat{N} = \frac{n_1 \times n_2}{m}$$

where $\hat{N}$ is the estimated population size, n_1 is the total caught in the first sample, n_2 is the total caught in the second sample, and m is the total caught in both samples (the recaptures).

8. Record the data in a table with space for entries for all the class data (Table 2 displays data from example simulations).

Three key points emerge. The first (commonly forgotten) is that trap success rates are often low in reality (e.g., Medina-Vogel et al., 2015), so using random number tables to determine trap success highlights this important point. The second point is that with low trapping intensity and poor trap success, it might not be possible to estimate population size at all. With five replicates at each trapping intensity, our own simulation data using this method were unable to estimate population sizes at a trapping intensity of 10 because no replicate included a recapture, whereas population estimates could be calculated with sampling intensities of 20 or 40 traps (Table 2). Instructors could, if they wished, introduce at this point a modified Lincoln index equation designed for use with small sample sizes (Krebs, 1999):

$$\hat{N} = \frac{(n_1 + 1) \times (n_2 + 1)}{m + 1}$$

Either way, the importance of sample size and trapping intensity will be clear. Finally, the estimated population sizes form a wide range at each sampling intensity (Table 2), reinforcing the point that the Lincoln index only estimates population size. If the class generates a large number of estimates, histograms of the sample size estimates can be drawn for the different sampling intensities and compared.

These points can be highlighted in class discussion. Students may comment that the replicate population estimates could be averaged to

Table 1. Template record sheet for recording the data from multiple trials. The first row is completed as an example.

Number of traps set	Cards Caught in First Sample (n_1)	Cards Caught in Second Sample (n_2)	Number of "Marked" Cards Caught in Second Sample (m)	Population Estimate[a]
40	2C, 8C, AC, 10D, QD, 7H, 9H, KH, 4S, JS (10)	6C, 8C, 10C, 2D, QD, AD, 3H, 10H, KH, 4S, 8S, JS (12)	8C, QD, KH, 4S, JS (5)	24
[etc.]				

[a]Calculate using Lincoln index (see text).

Table 2. Results from applying the simulation under three different sampling intensities (10, 20, and 40 traps), with five replicates under each condition. The 95% confidence intervals (CIs) are based on 95% binomial CIs for the proportion of recaptures in the second sample, using the Wald interval method (Zar, 2010, p. 546).

	10 traps set	20 traps set	40 traps set
Replicate 1			
n_1	2	15	12
n_2	8	10	31
m	0	2	11
$\hat{N}$	–	75	34
95% CI	–	34–313	23–64
Replicate 2			
n_1	3	11	17
n_2	3	14	31
m	0	1	6
	–	154	88
95% CI	–	53–173[a]	51–312
Replicate 3			
n_1	9	18	5
n_2	4	1	36
m	0	1	4
	–	18	45
95% CI	–	18–18	23–592
Replicate 4			
n_1	4	19	30
n_2	5	10	2
m	0	5	2
	–	38	30
95% CI	–	23–100	30–30
Replicate 5			
n_1	4	13	38
n_2	1	5	22
m	0	2	14
	–	32	60
95% CI	–	16–442	45–87

[a]In this case $m/n_2 < 0.1$ and $m < 50$, so strictly a Poisson estimate of the 95% CI is appropriate.

improve the overall estimate of population size. However, such a luxury is not possible in a real field study.

In most high school biology classes, the simulation would stop at this point because of time constraints. Undergraduate college classes, with more time, may proceed to the next exercise to estimate errors on population estimates.

○ Errors on Population Estimates

There are three approaches to estimating the 95% confidence intervals (CIs) for an estimate of population size derived using the Lincoln index (Krebs, 1999, p. 22):

- binomial confidence intervals, appropriate where the ratio of recaptures to total captures in the second sample m/n_2 exceeds 0.10;
- a normal approximation, used when the number of recaptured animals in the second sample exceeds 50; and
- Poisson confidence intervals, which should be used on all other occasions.

For the data in Table 2 where the population size could be estimated, the binomial confidence interval is appropriate for all cases except the second replicate at a sampling intensity of 20 traps. This replicate has $m/n_2 < 0.1$ and $m < 50$, so this is the only case where a Poisson estimate of the 95% CI is appropriate rather than a binomial one. We describe the procedure for calculating binomial confidence intervals, which applies to all other cases, below. Methods for applying the normal approximation and Poisson confidence intervals are given in Krebs (1999).

Estimating the population using the Lincoln index assumes that the proportion of marked animals trapped in the second sample is equivalent to the proportion of marked animals from sample 1 in the population as a whole. In reality, the proportion of marked animals in the second sample is an estimate of the proportion of marked animals from sample 1 in the population, and binomial 95% CIs can be calculated on that proportion (Zar, 2010, pp. 543–548). The upper and lower 95% confidence limits (CLs) on the proportion of recaptures can then be incorporated into Lincoln index calculations to estimate the upper and lower 95% CLs for the estimated population size. Students can work in the same groups as for the previous exercise. We suggest taking students through the procedure below, using the example data in step 3 below, and then asking them to proceed with their own data from the first exercise.

1. Calculate the proportion (p) of marked animals in the second sample:

$$p = \frac{m}{n_2}$$

2. Calculate the upper and lower binomial 95% CLs for the proportion in step 1. This can be done longhand using the formulae in Zar (2010, p. 543–548), by reading the chart in Krebs (1999, p. 27), or most simply by using interactive online freeware such as Epitools (http://epitools.ausvet.com.au/content.php?page=CIProportion&SampleSize=5&Positive=2&Conf=0.95&Digits=3) or VassarStats (http://vassarstats.net; choose proportions from the lefthand menu). A screenshot from the Epitools site shows the data entry and output for 31 animals in sample 2, 11 of which were marked in sample 1 (Figure 1). There are several different techniques for estimating the

Confidence limits for a proportion

Analysed: Sun Jun 07, 2020 @ 03:04 UTC

Inputs

Sample size	31
Number positive	11
Confidence level	0.95
CI method	Normal approx.

Results

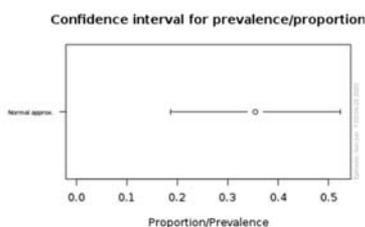|	Number positive	Sample size	Proportion/Prevalence	Lower 95 % CL	Upper 95 % CL
Normal approx.	11	31	0.355	0.186	0.523

CI plot

Figure 1. Calculating binomial confidence intervals for a proportion, based on the Wald method: example data entry and results output from the Epitools freeware site (http://epitools.ausvet.com.au/). Based on the data entered, the interpretation is that there is a 95% chance that the actual proportion of marked animals in the sample is between 0.186 and 0.523.

95% CIs (for a discussion, see Zar, 2010, p. 546), but for simplicity we use the Wald interval.

3. Calculate the upper 95% population estimate as

$$\hat{N}_{upper} = \left(\frac{1}{p_{upper}}\right) \times n_1$$

and the lower 95% population estimate as

$$\hat{N}_{lower} = \left(\frac{1}{p_{lower}}\right) \times n_1$$

For example, assume that $n_1 = 12$, $n_2 = 31$, and r (recaptures) = 11. The proportion of recaptures in the second sample is 11/31 = 0.355. Using the Epitools site, the binomial 95% CLs for this proportion are 0.523 and 0.186. Applying the equations from step 3, the 95% CI for the population estimate is 23–64.

Example data from sampling intensities of 20 and 40 traps (Table 2) show important points likely to arise in class. First, the 95% CIs are usually large and asymmetrical around the estimated population size. The exceptions are the third replicate at a sampling intensity of 20 traps and the fourth replicate at a sampling intensity of 40 traps, where the confidence interval is zero given that all the animals in sample 2 were recaptures (this is a consequence of using the Wald interval; alternative techniques available on the Epitools site would still have produced a range). Next, the second replicate at a sampling intensity of 20 traps gave a 95% CI of 53–173, not including the real population size of 52. Should this occur in class data, it affirms that 5% of calculated confidence intervals will not embrace the real population value. These points can be highlighted in class discussions of the confidence intervals, before moving to the final step of exploring assumptions.

○ Testing Assumptions

College students can be extended further by asking them to consider the consequences of violating some of the assumptions of the Lincoln index. The Lincoln index assumes the following:

1. The population is unchanged during the study, with no births, deaths, or migration.

2. On each sampling occasion, all animals have an equal likelihood of being caught. In the second sample, this requires that marking does not change the likelihood of capture, including the possibility that previously caught animals are more cautious (trap shy) or more likely to return to a trap, perhaps to eat a bait (trap happy).

3. Animals neither gain nor lose marks, and all marked animals are identified correctly in the second sample. Gaining a mark might be possible if mutilation marking is used (e.g., an animal may have an ear notched in a fight, not by an ecologist), while tags may be lost.

These assumptions can be tested by simple adjustments to the basic procedure. For example, trap shyness can be simulated by consulting random number tables for each marked animal recaptured in the second sample. If the random number is even, the animal is caught. If it is odd, the animal avoided the trap and is not counted as a recapture. A second example is violating the assumption that all animals have an equal likelihood of being caught on either sampling occasion. The possibility that females are more cautious and therefore less likely to be caught can be simulated by calling all black cards males and all red cards females. If a female card is selected, random number tables are consulted and the animal is considered caught only if the number is even. No such correction applies to the male cards. Many other possibilities are possible, giving students the opportunity to decide for themselves what assumption to violate and explore the consequences.

Ideas for testing can be developed in class discussions. Groups can then test the consequences of violated assumptions by modifying the basic procedures.

○ Assessment

Many different assessment strategies are possible. A conventional laboratory report is one option, or one group member could complete a conventional laboratory report, a second a conference poster, a third an online blog, and a fourth a spoken presentation. If students complete several group projects over a semester, they can take a different approach with each report and practice several communication styles.

Taking the conventional laboratory report as one example, questions that students could be asked to answer in the discussion include the following:

- Does the number of traps set make a difference to the population estimates? (Students should find that setting a small number of traps makes it difficult to estimate a population size at all.)

- How often did the 95% CI for an estimated population size include the real population size of 52? (Hopefully, most times. However, on rare occasions the real population may lie outside the 95% CI.)

- Why is it important to present population estimates together with the 95% CI? (If multiple groups have estimated the population, then students should see clearly that estimates can vary widely. Calculating the 95% CI acknowledges that uncertainty.)

If students designed a violation of one of the assumptions of the Lincoln index and estimated the population size after the violation, they could be asked in the introduction to their reports to predict the consequence of the violation. Then, in the discussion, they could answer these questions:

- Following the violation, did the population estimation increase, decrease, or remain about the same?

- How does your answer in question 1 compare to your prediction about what would happen after the violation?

- How important is it to understand the basic biology of a study species *before* beginning a capture–recapture study?

- How would you design a study to estimate population size if you knew in advance that males were much more likely to be trapped than females?

Students could also be required to answer some or all of these questions if they were taking the alternative approach of a conference poster, an online blog, or a spoken presentation. However, in these cases, marks would need to be allocated to unique features of each mode of presentation. All approaches would strengthen students' understanding of scientific method and their critical thinking. Depending on the kind of report requested (formal written report, blog, poster), students would gain experience in different communication styles.

○ Classroom Application

We ran a version of this exercise with 11th-grade students (mainly 16 years old) in a Western Australian biology class in 2014, concentrating on estimating population size and calculating 95% CIs. Students estimated population sizes using the Lincoln index and determined 95% CIs using the chart in Krebs (1999). Estimating population sizes was not a problem, but estimating confidence intervals from the chart was cumbersome, so the online calculators are a better option.

○ Points Transferable to Other Teaching Environments

While this article has focused on the Lincoln index as an example of capture–recapture methods of population estimation, there are general points applicable to many teaching situations, including these:

- Confidence intervals are valuable in interpreting estimated values because they show the range of possibilities, not just a single point.

- Many analyses make assumptions about the subjects under study or the numerical properties of the data collected. If these are violated and no adjustments are made to compensate, incorrect conclusions may be drawn.

- The suggestion to have different group members prepare a conventional laboratory report, a conference poster, an online blog, or a spoken presentation gives experience in different communication styles. It also allows group members to collaborate in collecting data but then submit independent work, thereby avoiding the common complaint of carrying passengers on group reports.

○ Acknowledgments

○ References

Bishop, J.A. (1979). The dynamics of the Liverpool taxi-cab population 1971–78. *Journal of Biological Education*, *13*, 192–194.

Bishop, J.A. & Bradley, J.S. (1972). Taxi-cabs as subjects for a population study. *Journal of Biological Education*, *6*, 227–231.

Borchers, D. (2012). A non-technical overview of spatially explicit capture–recapture models. *Journal of Ornithology*, *152*(Supplement 2), 435–444.

Calver, M.C., Porter, B.D. & Bradley, J.S. (1990). A simple simulation for teaching capture–recapture methods of population estimation. *Journal of Biological Education*, *24*, 267–272.

Gilhooley, E. (1985). Using shopping trolleys to teach animal population estimating techniques. *Journal of Biological Education*, *19*, 184.

Goetze, J.R. & Rodriguez, M. (2018). A "sweet" activity to teach basic population estimation principles, community diversity assessment, and mathematical reasoning to biology students. *American Biology Teacher*, *80*, 522–528.

Hunter, C.M., Caswell, H., Runge, M.C., Regehr, E.V., Amstrup, S.C. & Stirling, I. (2010). Climate change threatens polar bear populations: a stochastic demographic analysis. *Ecology*, *91*, 2883–2897.

Krebs, C.J. (1999). *Ecological Methodology*, 2nd ed. Menlo Park, CA: Benjamin Cummings.

Marlow, N.J., Thomas, N.D., Williams, A.A.E., Macmahon, B., Lawson, J., Hitchen, Y., et al. (2015). Lethal 1080 baiting continues to reduce European red fox (*Vulpes vulpes*) abundance after more than 25 years of continuous use in south-west Western Australia. *Ecological Management and Restoration*, *16*, 131–141.

Masene, R.S. (2001). A simulation game showing the capture–recapture method of population estimation prior to field investigation. *Science Activities*, *38*, 21–24.

Medina-Vogel, G., Barros, M., Monsalve, R. & Pons, D.J. (2015). Assessment of the efficiency in trapping North American mink (*Neovison vison*) for population control in Patagonia. *Revista Chilena de Historia Natural*, *88*(1).

Mudie, K. & Brotherton, J.(Eds.) (1984). *Core Biology, Practical*. Sydney: Sofima.

Musseau, C., Boulenger, C., Crivelli, A.J., Lebel, I., Pascal, M., Boulétreau, S. & Santoul, F. (2015). Native European eels as a potential biological control for invasive crayfish. *Freshwater Biology*, *60*, 636–645.

Naves, L.C. (2018). Geographic and seasonal patterns of seabird subsistence harvest in Alaska. *Polar Biology*, *41*, 1217–1236.

Steiner, A.J. (1983). Comtrap: a trapping simulation interactive computer program. *Journal of Wildlife Management*, *47*, 361–367.

Whiteley, A.R., Woolf, J., Kennedy, K., Oberbillig, D. & Brewer, C. (2007). Classroom mark–recapture with crickets. *American Biology Teacher*, *69*, 292–297.

Zar, J.A. (2010). *Biostatistical Analysis*, 5th ed. Upper Saddle River, NJ: Prentice Hall.

MICHAEL CALVER is a Professor of Environmental and Conservation Sciences at Murdoch University, Murdoch, Western Australia, Australia 6150; e-mail: m.calver@murdoch.edu.au. TIMOTHY BLAKE is a Biology Teacher at Trinity College, East Perth, Western Australia, Australia 6004; e-mail: blake.tim@trinity.wa.edu.au.