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Chapter 11

Introduction to Mark-Recapture Census Methods Using the Seed Beetle, *Callosobruchus maculatus*

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Introduction

Population size, or the abundance of organisms in a study site, is the most fundamental of the primary demographic statistics. Here, we present a laboratory study that introduces college undergraduates to mark-recapture methods that estimate population size. At Morehouse College, students conduct this study in a junior/senior level ecology course, but the study can easily be modified for students at the introductory level. The level of mathematical sophistication in this exercise is low, requiring only that students perform simple algebra. Students apply a simple mark-recapture technique to estimate population size in cultures of a seed beetle, *Callosobruchus maculatus*. After completing this study, students not only gain rudimentary knowledge of statistical methods, e.g., standard deviation and 95% confidence limits, but also learn how to assess the reliability of population-size estimates.

Student Outline

Objectives

1. Learn the mathematical methodology for estimating population size in nature by means of mark-recapture techniques.
2. Perform an experiment to estimate the number of adults in a series of bean beetle cultures and to evaluate the reliability of the estimations.

Introduction

Estimating the abundance of organisms, especially in naturally occurring populations, is a fundamentally important activity in ecological research. An accurate census informs us of changes in population size due to migrations into or out of our sampling sites, as well as to season-related recruitment (births) and mortality (deaths). There are many ways to perform a population census, and each method makes certain assumptions about the population. In this study, we will use a census method known as "mark-recapture" and learn about those assumptions.

Mark-recapture techniques allow ecologists to track movement of individuals in space and in time through a population. Data from mark-recapture experiments are so important that researchers continue refining analytical techniques to maximize the information yield from mark-recapture data (Lebreton et al., 1992; Schwarz and Seber, 1999; Schtickzelle et al., 2003). Here, we briefly describe three basic mark-recapture methods: the Petersen, the Schnabel, and the Jolly-Seber methods.

The Petersen method, also known as the Lincoln index (Haag & Tonn, 1998), is the easiest of the mark-recapture census methods to perform because it is based on single episode of marking and recapturing individuals (Table 1). The important assumptions of the Petersen method are:

1. The population being sampled is closed (no births/deaths/migration) so that population size remains constant throughout the sampling period.
2. Every individual has the same chance of being caught; in other words, sampling is random.
3. Marks are not lost in the interval between mark and recapture.

Population estimation with the Petersen method is based on equivalent ratios such that the proportion of the population that is marked and released will be the same as the proportion of individuals in a recapture sample that were previously marked:

$$\frac{\text{Total number marked in population (M)}}{\text{Total number estimated in population (N)}} = \frac{\text{Number found marked in recapture sample (R)}}{\text{Total number in recapture sample (C)}}$$

In contrast, the Schnabel method requires successive episodes of recapture; yet, like Petersen, the Schnabel method requires only a single episode of marking (Table 1). That is, individuals are marked at first capture, and no further marking is required even with subsequent recaptures. While it makes the same assumptions as in Petersen, the Schabel method's reliance on multiple sampling episodes makes it particularly sensitive to violations of the assumptions noted in the Petersen method.

Often, ecologists cannot study closed, constant-sized populations. The Jolly-Seber (JS) method was developed specifically to study demographic patterns of natural, or open, populations. Like Schnabel, the JS method involves successive episodes of capture. However, JS also requires that the census-taker keep track of when an individual from a study population was last caught, i.e. at the very least, marks must correspond to a unique time of capture for each recaptured individual. Although the most challenging logistically and mathematically, the JS method is also the most informative of the three mark-recapture census methods described thus far: JS estimates not only population size, but also persistence rate (i.e. survival and site fidelity combined) from a group of marked individuals. Aside from the assumption of an open population, the JS method assumes that:

1. Every individual has the same chance of being captured at each sampling.
2. Marks are not lost during the entire census period.

Table 1. Summary of three basic mark-recapture census methods.

Method	# of recapture events	# of marking events
Petersen	single	single
Schnabel	multiple	single
Jolly-Seber	multiple	multiple

The Importance of Randomness

A central element in all three mark-recapture census methods is the notion that populations are sampled in random fashion. However, what exactly is "random?" For our purposes, randomness means that each individual in the study population has the same (or nearly the same) probability of being captured, so that each sampling event has no effect on previous sampling events. In applying Petersen mark-recapture techniques, we not only presume that (1) recapture rate reflects the underlying spatial distribution in the study population, i.e. homogeneously distributed individuals, but also assume (2) our manner of sampling is itself random. Violation of either of these assumptions leads to biased estimates of population size.

On Reliability of Estimated Counts

Regardless of census method, ecologists should always evaluate the reliability of their population-size estimates. Why? An estimate, by definition, carries with it a level of uncertainty, so that one population-size estimate could very well be as (un)informative as another could. Hence, instead of relying on a single estimate of population size, ecologists construct a range of estimates known as a "confidence interval." A confidence interval of our estimated population size (N_{EST}), is a numerical range within which the actual, or true, population size (N_{TRUE}) will fall with a certain level of probability (Sokal and Rohlf, 1981). For example, a 95% confidence interval of N_{EST} is one in which the experimenter specifies the width of a series of confidence intervals, such that 95 of 100 intervals contain N_{TRUE}. Population-size estimates belonging to the same confidence interval have an equal chance of representing the true population size. In ecology, the *de facto* standard level for confidence intervals is 95 per cent, i.e. a 95% confidence interval fitted around our point estimate of population size.

In this study, you will apply the Petersen method to obtain point estimates of population size in a stock of cowpea seed beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). You will then

evaluate their reliability by fitting 95% confidence intervals around those point estimates, and later comparing your point estimates against the actual number of beetles in your study population.

Bean beetles, *Callosobruchus maculatus*, are agricultural pest insects originating from Africa and Asia. Females lay their eggs singly on the surface of beans (Family Fabaceae). After several days, the beetle larva hatches out and burrows into the bean. At 30 °C, pupation and emergence of an adult beetle occurs 25-30 days after oviposition. Adults mature 24-36 hours after emergence, and they appear not to feed. Adults may live for about 12 days, in which time females mate and oviposit. Brown and Downhower (1988) provide more information on the natural history of *C. maculatus*.

Methods

Each group of students will receive a petri dish filled with a single layer of mung beans and some beetles. Each group should mark a pre-determined number of live beetles in the colony dish provided, e.g. mark 20 or more live beetles – ignore the dead ones – and then use a flat toothpick to apply nail polish to that number of beetles. Apply a small drop (or dot) to the back of the beetle's thorax, and avoid painting the wing covers since *C. maculatus* beetles are agile enough to wipe off paint from this area. Divide the counting and marking workload equally among group members. Note the exact number of beetles that you've marked, and then allow marked beetles to hide ("disperse") among the mung beans and unmarked individuals in the colony dish. You and your lab partner(s) are now ready to estimate population size in your colony dish.

Using soft forceps, each person in your group should be allowed exactly two minutes to withdraw randomly as many beetles (both marked and unmarked) as possible from your group's petri dish. At the end of each person's sampling, count the number of marked and unmarked beetles that were withdrawn, and then return the sampled beetles to the petri dish. Each person in your group should repeat the sampling from this same petri dish. You can check the accuracy of your counting by noting that the number of marked and unmarked beetles should sum to the total number of beetles captured (Table 2).

Table 2. A template for tallying each person's count.

Name of person sampling	
Total number of beetles captured (C)	
Number of marked beetles captured (R)	
Number of unmarked beetles captured	

Data Analysis

Estimate the total number of beetles in your study population. Use the following symbols to organize your data (after Krebs, 1989):

M = number of individuals marked and released
C = total number of individuals captured (in the recapture sample)
R = number of individuals in the recapture sample that are marked
N_{EST} = your estimate of the total population size

As you already know, $\frac{M}{N_{EST}} = \frac{R}{C}$, so that the reciprocal, $\frac{N_{EST}}{M} = \frac{C}{R}$, is also true. We can then re-arrange the equation to obtain the estimated total population size: $N_{EST} = \frac{M \times C}{R}$.

This calculation can be performed in a Microsoft™ Excel™ spreadsheet titled "PopEst.xls" located on the Ecology laboratory computers. PopEst.xls will also calculate the 95% confidence limits for your point estimate of total population size (see Appendix A).

Once you have made your calculations, you and the members of your group will need to assess the accuracy (as distinct from precision, and explained in Appendix C) of your population-size estimates. To determine accuracy of your population-size estimates, you should first count every single living beetle (both marked and unmarked) in your colony dish and then compare that number with your calculated estimate(s).

Points to Ponder

After you have finished counting, consider the following questions:

1. Among the counters in your class, which person's point estimate was closest to the actual population size? Which person's estimate was farthest?
2. Among the counters in your class, which person's confidence interval was the narrowest? Which person generated the widest 95% confidence interval?
3. Identify any specific factors in your class's counting method that may have compromised the validity of your estimate of population size.
4. Which person and which group obtained the "best" estimate of population size? (The class as a whole should decide on criteria for "best" estimate.)
5. Of the three variables—that is, C, M, and R—required to obtain a Petersen estimate of population size (N_{EST}), which variable ought to be maximized? Please explain your conclusion.

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Appendix A: Building Poisson 95% Confidence Intervals

Poisson 95% confidence intervals are based on a Poisson discrete frequency distribution, described mathematically as follows:

$$P_R = \frac{\mu^R}{R!} \cdot e^{-\mu} ,$$

where μ is the true mean number of marked organisms in the 2nd (recapture) sample, R is the number of recaptured (marked) beetles, *e* is Euler's number (or base of the natural logarithm = 2.78128…), and P_R is the probability of recapturing a (marked) beetle. Since our interest is in two-tailed 95% confidence intervals, P_R is 0.025 and 0.975. To find the Poisson 95% confidence interval for our observed R, we would need to solve the above equation to obtain theoretical values of R that correspond to the upper and lower 95% confidence limits.

Fortunately, statisticians saved us from all that hassle. The following table (modified from Appendix 1.2 of Krebs [1989]) provides the lower and upper Poisson 95% confidence limits for an observed R, or the number of recaptured beetles, in the companion spreadsheet, "PopEst.xls."

To construct Poisson 95% confidence intervals for your population-size estimate, look in the table below and along the column labeled "R" for the number corresponding to your observed R. The two columns to the right of each R in the table correspond to the lower and upper limits of your observed R. Enter these lower and upper R values one-at-a-time in the PopEst.xls spreadsheet. Excel will then calculate N_{EST} values at the upper and lower 95% confidence limits, using the Petersen population estimation equations.

R	Lower	Upper	R	Lower	Upper	R	Lower	Upper	R	Lower	Upper
1	0.051	5.323	**26**	16.77	37.67	**51**	37.67	66.76	**76**	58.84	94.23
2	0.355	6.686	**27**	17.63	38.16	**52**	38.16	66.76	**77**	60.24	94.70
3	0.818	8.102	**28**	19.05	39.76	**53**	39.76	68.10	**78**	61.90	96.06
4	1.366	9.598	**29**	19.05	40.94	**54**	40.94	69.62	**79**	62.81	97.54
5	1.970	11.177	**30**	20.33	41.75	**55**	40.94	71.09	**80**	62.81	99.17
6	2.613	12.817	**31**	21.36	43.45	**56**	41.75	71.28	**81**	63.49	99.17
7	3.285	13.765	**32**	21.36	44.26	**57**	43.45	72.66	**82**	64.95	100.32
8	3.285	14.921	**33**	22.94	45.28	**58**	44.26	74.22	**83**	66.76	101.71
9	4.460	16.768	**34**	23.76	47.02	**59**	44.26	75.49	**84**	66.76	103.31
10	5.323	17.633	**35**	23.76	47.69	**60**	45.28	75.78	**85**	66.76	104.40
11	5.323	19.050	**36**	25.40	48.74	**61**	47.02	77.16	**86**	68.10	104.58
12	6.686	20.335	**37**	26.31	50.42	**62**	47.69	78.73	**87**	69.62	105.90
13	6.686	21.364	**38**	26.31	51.29	**63**	47.69	79.98	**88**	71.09	107.32
14	8.102	22.945	**39**	27.73	52.15	**64**	48.74	80.25	**89**	71.09	109.11
15	8.102	23.762	**40**	28.97	53.72	**65**	50.42	81.61	**90**	71.28	109.61
16	9.598	25.400	**41**	28.97	54.99	**66**	51.29	83.14	**91**	72.66	110.11
17	9.598	26.306	**42**	30.02	55.51	**67**	51.29	84.57	**92**	74.22	111.44
18	11.177	27.735	**43**	31.67	56.99	**68**	52.15	84.67	**93**	75.49	112.87
19	11.177	28.966	**44**	31.67	58.72	**69**	53.72	86.01	**94**	75.49	114.84
20	12.817	30.017	**45**	32.28	58.84	**70**	54.99	87.48	**95**	75.78	114.84
21	12.817	31.675	**46**	34.05	60.24	**71**	54.99	89.23	**96**	77.16	115.60
22	13.765	32.277	**47**	34.66	61.90	**72**	55.51	89.23	**97**	78.73	116.93
23	14.921	34.048	**48**	34.66	62.81	**73**	56.99	90.37	**98**	79.98	118.35
24	14.921	34.665	**49**	36.03	63.49	**74**	58.72	93.48	**99**	79.98	120.36
25	16.768	36.030	**50**	37.67	64.95	**75**	58.72	93.48	**100**	80.25	120.36

Appendix B: Correction for Small Sample Size

Krebs (1989, Ch. 2) provides a way to correct for a theoretically upwardly biased population-size estimate. This bias can be quite significant with small populations, or rather when the sum of marked animals and the number of animals in the 2nd (recapture) sample is greater than the actual population size: (M+C) > N_{TRUE} (Krebs, 1989: 17). Instead of using $N_{EST} = \frac{M \times C}{R}$, add 1 to each of the terms on the right side of the equation, and then subtract 1 from the quotient: $N'_{EST} = \frac{(M+1) \times (C+1)}{R+1} - 1$. If you elect to use the bias-corrected N, you should also make the corresponding changes in the "PopEst.xls" file.

Appendix C: Accuracy Versus Precision

While counting might seem like a straightforward academic exercise, obtaining a reliable count is an important skill to have, particularly when conducting a population census. A reliable population size estimate is one that minimizes bias by maximizing both accuracy and precision:

high reliability = low bias = (high accuracy + high precision).

Accuracy addresses the proximity, or "near-ness," of a point estimate to the true value of population size. For example, if the true abundance of animals in a study population is 119, then a point estimate of 150 animals is more accurate than one of 190. In most real-world cases, we cannot count every individual in our study population; and without knowledge of the true population size, we cannot evaluate accuracy of a population estimate. We are then left with the second, more useful parameter of reliability – precision.

Because they depend on statistical variance, measures of precision address uncertainty in a population estimate. The greater the statistical "noise" around a point estimate, the wider the error bars that we must fit around that estimate, and the more choices of point estimates that could correspond to the actual population size. For example, in our census study, we build 95% confidence intervals to obtain a range of values that have equal probability of representing the true total number of beetles in our study population. More importantly, such confidence intervals allow us to eliminate values from an infinite universe of population-size estimates, since values lying beyond the limits of our confidence interval are less likely to correspond to the true population size compared to values falling within the confidence limits. In a counter-intuitive yet scientific sense, confidence intervals tell us what population size most likely is not! Therefore, unlike with accuracy, we can and should always evaluate precision of our population-size estimates; and we can evaluate precision – and, hence, reliability – of our estimates with confidence intervals.

Materials (one per student group, unless noted otherwise)

- bottle of quick-drying nail polish, e.g. Revlon™ Swoop 260 (orange-red) or some other brightly colored nail polish
- bottle of nail-polish remover or isopropyl (=rubbing) alcohol
- paper towels and/or Kimwipes®
- mechanical clicker counter
- stopwatch or countdown timer
- pair of soft forceps, e.g. Bioquip™ featherweight forceps (Catalog No. 4748), one per student
- organically grown mung beans (*Vigna radiata*), about 1/3 cup
- 150x25-mm plastic petri plate and cover
- 100-200 adult beetles (*Callosobruchus maculatus*)
- computer with Microsoft™ Excel™ (for data analysis)

Notes to the Instructor

Culturing *Callosobruchus maculatus*

Mung beans should be pesticide-free and free of any bean-burrowing insects. We purchase pre-packaged, organically grown mung beans from a local natural foods store.

We initiate colonies of *C. maculatus* beetles by placing about 10 males and 10 females into a 150x25-mm petri dish covered with a layer of mung beans. Laboratory stocks are kept on a 12-hour daily light cycle (DLC) and at 25°C year-round. Every two months, we establish new colony plates with individuals from existing colonies and fresh mung beans (for oviposition). Cultures should be started two months in advance of expected use for this study to ensure sufficient numbers of adult beetles. The ideal number of adults in a culture dish for this study is 100-200.

Recently, we experimented with alternative rearing environments. By isolating and incubating eggs at 14-hour DLC and 30°C, we obtained acceptable egg-to-adult survival (approx. 65% across sexes) and, more importantly, predictably short egg-to-adult development time (Table 3).

Table 3. Summary statistics on the life cycle of unmated *C. maculatus* individuals cultured and maintained in isolation.

Sex	Mean ± SE (sample N) Egg-to-Adult Development Time @ 30°C	Mean±SE (sample N) Adult Longevity @ 25°C
Virgin Female	28.1 ± 1.39 (22) days	25.1 ± 0.74 (54) days
Virgin Male	28.6 ± 0.38 (31) days	21.1 ± 0.57 (62) days

Because *C. maculatus* adults appear to abstain from normal feeding (Messina, 1991; Fox et al., 1995; Eady et al., 2000; but see Fox, 1993), males and females cannot replenish nutritional stores spent during copulation and oviposition. With cumulative loss of mass, beetles allowed to mate and oviposit have lifespans closer to 14 days post-eclosion (Olvido and Blumer, unpublished data). Thus, when kept in colony plates, egg-to-egg generation time of *C. maculatus* can be as short as 27 days. Population sampling should take place within 12 days after the first adults have emerged.

Marking Techniques

The process of marking *C. maculatus* individuals presents several logistical challenges, one of which stems from the insect's small size (approx. 2 mm anterior-posterior length). Students can use the brush applicator that is included with each bottle of nail polish, provided you (i.e. instructor) trim the brush to a few hairs. Alternatively, we recommend you substitute a flat toothpick for a fine-point brush when marking *C. maculatus* individuals.

Once you have decided on the appropriate marking tool, you should apply a small but visible mark on or as near as possible to the beetle's thorax. It is best to have students aim for the white dot on the thorax of *C. maculatus*, and to caution students against applying marks that may hinder the natural mobility of the insect. For example, instruct students to avoid painting over the beetle's head.

A number of participants at the A.B.L.E. 2004 workshop had trouble applying nail polish to *C. maculatus*. To address these issues, the workshop participants suggested alternative methods for marking these small animals. Below, we summarize the main advantages and disadvantages of different techniques for marking *C. maculatus* and other small terrestrial arthropods, and encourage instructors to experiment with each method (Table 4). By no means is this list exhaustive, so we also encourage instructors to send us their comments on other alternatives for inclusion in future revisions of this laboratory study.

Another logistical challenge in marking *C. maculatus* stems from the insect's relentless effort to hide, especially when perturbed. The more active the beetles are, the more difficulty students will have in applying marks to the beetle's thorax. If cooling the entire classroom to 20°C seems impractical, then you might try cooling only the petri dish containing the sample of beetles. For example, you could place the population dish in a refrigerator (4°C) for about 5 minutes just before marking them. Alternatively, you could place the population dish on a flat bed of ice cubes during the marking period.

While *C. maculatus* is a hardy and durable insect, students should handle these beetles carefully. We use BioQuip™ featherweight forceps because they allow for a firm grip of insect specimens without injuring them. Surprisingly, students still manage to maim – and, in a few cases, accidentally crush – beetles when using soft-grip forceps. Hence, we strongly recommend that you remind students to squeeze the forceps as near as possible to the pivot point joining the two arms of the forceps before allowing students to handle beetles. As an alternative to using forceps, students might consider using a medium- or fine-point brush to separate beetles from mung beans during the counting period.

Table 2. Summary of four basic marking techniques for *C. maculatus* adults.

Type of Marking	Brief Description	Advantages	Disadvantages
(1) Quick-drying paint	• described above	• paint dries quickly and permanently; small chance of marking non-target animals	• paint may dry too quickly, and marked animal may get fixed to a substrate or another animal; students are likely to smother target animal with paint, thus hindering mobility; clean-up can be challenging
(2) Felt-tip pen	• like (1), but marks are applied with lightly colored felt-tip pen	• more precise and less messy than (1) in the application of marks	• difficult to see mark; some aromatic inks, e.g. in xylene-based pens, may prove toxic to insects (though C. maculatus seems to tolerate marks from our MonAmi® alcohol-based pen)
(3) Fluorescent dust	• target animal thrown into a shallow bath of fluorescent dust, and then allowed to walk off the excess dust from itself	• requires minimal effort in marking, and easier to implement than either (1) or (2)	• great potential for confusing marked and unmarked animals, as dust easily transfers to non-target animals; high potential for losing marks
(4) "Invisible" dust	• target and non-target animals thrown into separate baths of invisible dust types, both of which appear white under visible light but differently colored under UV-A, or "black light"	• same as in (3), with additional advantage that marks have equal effect on both target and non-target animals; novelty and "pop culture" appeal of black lights	• same as in (3); dust marks can easily be exchanged between target and non-target animals; possible safety issues when working with UV light sources

Containment Issues

The cover of a colony petri dish (or any high-walled container) provides a useful container for temporarily housing captured beetles. Essentially, students count as a working pair—one to transfer beetles from the population dish to the plastic holding container, and the other to keep captured beetles inside the container. It is important to emphasize to students in each working pair that they should not sample the population dish at the same time; rather, one and only one student should focus on sampling beetles from the population dish while the other student focuses on beetle containment and recording counts. Later, the students in that working pair switch duties, so that both can gain experience with each aspect of sampling.

Adult *C. maculatus* seem averse to open environments and flat surfaces (A.E. Olvido, personal observation), so you can easily contain counted beetles by providing them with several mung beans to which they can cling and hide beneath. You should instruct students to keep beetles in the holding container by using a flat toothpick (or other pointed object or dry paint brush) to dislodge beetles crawling on or near the rim of the container. We have found it useful to smear a generous amount of Vicks™ petroleum gel around the inside rim of the holding container; we wipe away as

much of the gel from the rim of the holding container before returning counted beetles to the population dish.

Probable Causes of Sampling Bias

An important assumption in any mark-recapture method is random sampling. As explained in the student outline, the hallmark of random sampling is independence of sampled events: Students sample randomly when they capture a beetle (marked or unmarked) without significantly affecting the probability of subsequent captures of marked or unmarked beetles, leading to unbiased estimates of population size. However, results from the A.B.L.E. 2004 workshop show that the Petersen technique as applied in this study generally yields downwardly biased point estimates of population size (Fig. 1). A possible cause of this bias may be non-random sampling, such as when students use the visible marks to capture beetles, which results in a higher-than-expected recapture rate. To address the hypothesis that census marks act as visual cues that significantly increase recapture rates (hence, downwardly biasing population-size estimates), we completed a simulated population census using inanimate Lego™ blocks.

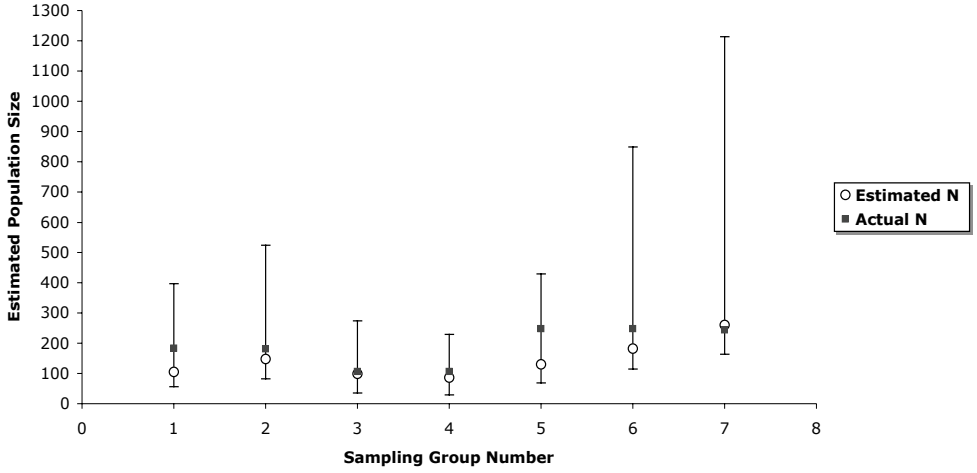

Figure 1. An evaluation of bias in Petersen estimates of population size by A.B.L.E. 2004 workshop participants. Error bars indicate Poisson 95% confidence intervals.

Our simulated population consisted of exactly 624 Lego™ blocks with various colors and shapes (Table 5):

Table 5. Composition of our Lego™ population.

	1x2	1x3	1x4	1x6	2x2	2x3	2x4	Total
BLUE	46	20	32	6	24	10	18	156
RED	46	20	32	6	24	10	18	156
WHITE	46	20	32	6	24	10	18	156
YELLOW	46	20	32	6	24	10	18	156
Total	184	80	128	24	96	40	72	624

We then selectively applied marks to an individual series of Lego™ blocks of different colors and shapes, so that the exact probability of recapture in this population was known for both control (i.e., blind sampling) and experimental treatments (i.e. color and/or shape visual cues). After several rounds of sampling, we obtained results confirming that visually guided sampling can result in higher-than-expected recapture rates, and consequently lower estimates of population size (Fig. 2).

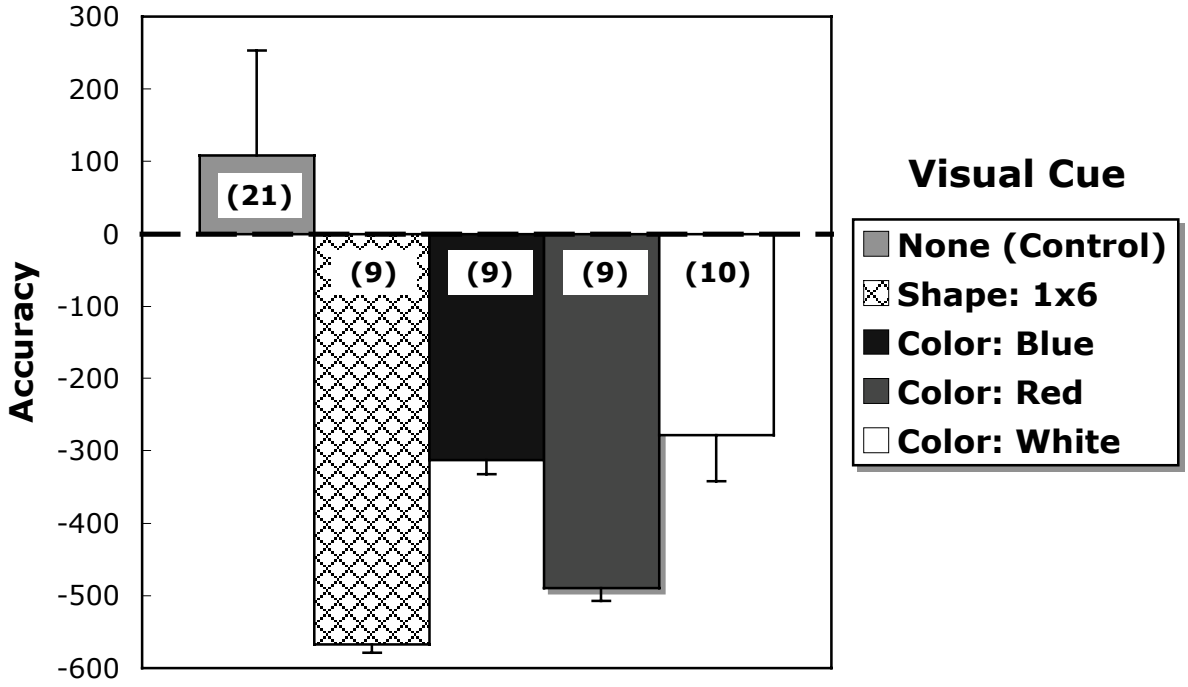

Figure 2. Visually guided sampling yields downwardly biased estimates of population size in a simulation of Petersen mark-recapture sampling ($F_{4,53} = 5.88$, $P < 0.001$, 1-beta = 0.981). Mean accuracy (+/- 1 SE) was measured as the difference between estimated population size (N_{EST}) and actual population size (N_{TRUE}). Numbers in parentheses indicate number of non-zero N_{EST} for that treatment.

Aside from random sampling, the Petersen technique and other mark-recapture census methods also assume equal sampling effort between mark and recapture samples. In this study, we allow students only 2 minutes to capture and count beetles. However, there are other ways to standardize sampling effort. Instead of a 2-minute sampling window, for example, students can opt to sample by absolute count, such that the number of beetles they mark and release (i.e., the 1st sample, or "M" group) equals the number of beetles that they subsequently catch (in the 2nd sample, or "C" group). A third type of sampling method – yet untested – would be to sample beetles by volume: Students use a measuring spoon to scoop up a mixture of beetles and beans of a standard volume for both 1st and 2nd samples. This "volume sampling" method, of course, presumes a homogeneous distribution of marked beetles.

Using Excel™, we calculated N_{EST} for various combinations of M, C, and R to illustrate the relationship between sampling effort, recapture rate, and accuracy. Given our simulated population of exactly 624 Lego™ blocks, we can ask "From the Petersen equation [shown in the student outline], what is the combination of M, C, and R that generates the most accurate N_{EST}?" The answer appears as a series of M-C-R combinations (Fig. 3).

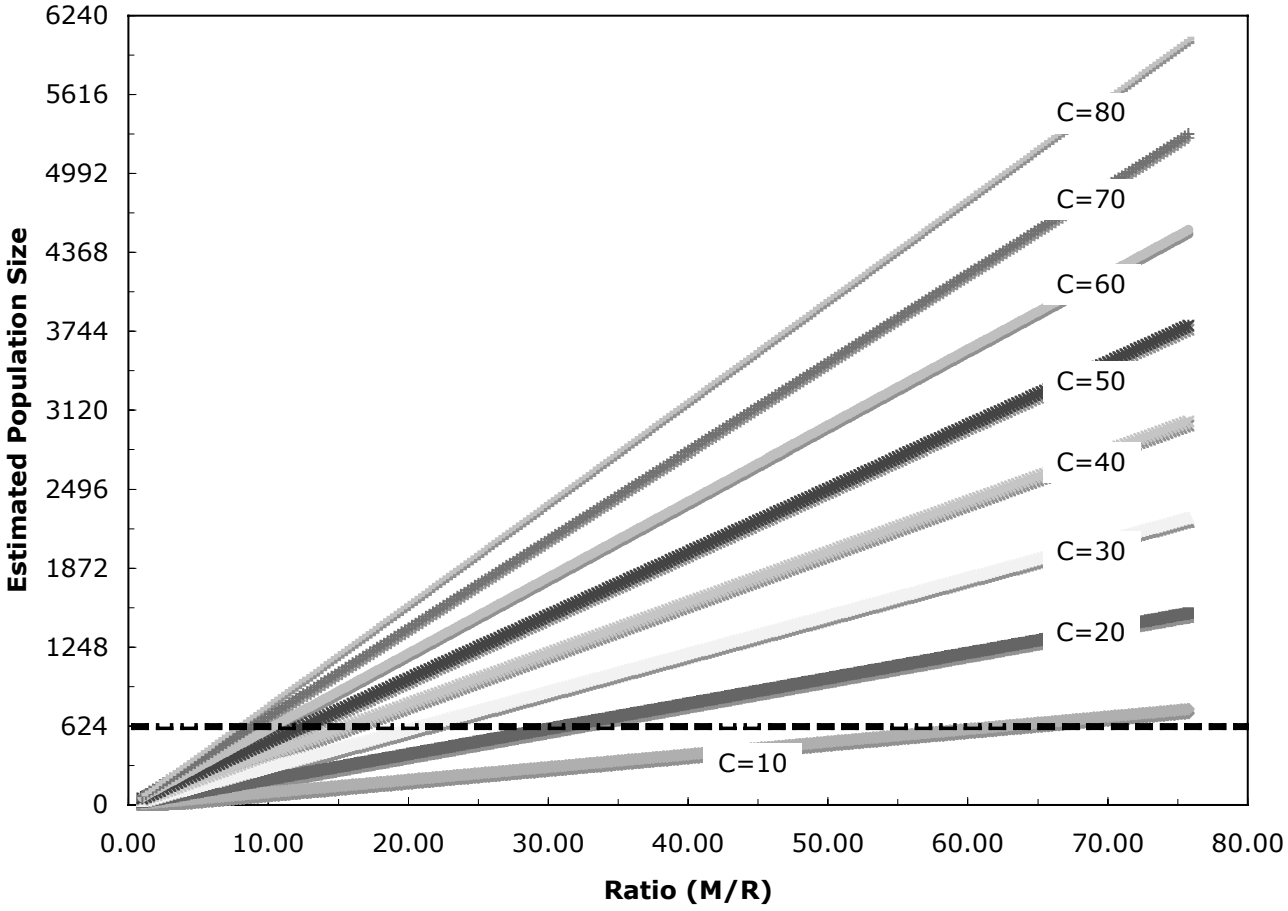

Figure 3. A graphical guide to sampling effort based on simulations of Petersen population-size estimation. The horizontal dashed line indicates the known population size.

Knowing the precise relationship among the variables in the Petersen equation will guide you in adjusting the written protocol for this study to suit your students' sampling abilities and to work with classroom time constraints. For example, let's assume a true population size of 624 beetles, i.e., the same as our population of Lego™ blocks: If students demonstrate a certain facility in capturing *C. maculatus* adults, you might suggest that students strive to mark and release 50 beetles (M=50), then randomly sample 50 beetles (C=50). A ratio of marked-to-recaptured beetles (M/R) that yields the most accurate N_{EST} would then be approximately 13.0, or one recapture for every 13 beetles originally marked and released (Fig. 3). Thus, a sample size of 50 for both M and C groups would suffice in possibly obtaining accurate estimates of population size, whereas a sample size of less than

13 guarantees an inaccurate estimate. Note that absence of recaptures precludes estimation of population size (Fig. 3).

If, on the other hand, students show less manual dexterity (or self-motivation), you might suggest that they mark and release only 30 beetles (M=30), and later sample 30 beetles (C=30). Depending on how randomly they sampled, you should expect an M/R ratio of approximately 22 (one recapture per 22 beetles originally marked and released) for a reasonably accurate population-size estimate (Fig. 3).

One Final Tip

As indicated in the student outline, students assess accuracy by counting all living beetles in their population dish. You can facilitate accurate counts by using a sieve to separate beetles from mung beans. At Morehouse College, we've fashioned our own sieves by hand-drilling 1/8-inch holes in 150 x 25 cm petri dish covers. You might be able to save yourself time and effort by purchasing wire-mesh sieves from vendors that offer soil analysis tools, e.g., Carolina Biological or Ben Meadows. Sieve designations of Nos. 5, 6, or 7 – corresponding to diameter openings of 4.00 mm, 3.35 mm, and 2.80 mm, respectively (consult URL *http://www.wovenwire.com/reference/screen-sieve-pr.htm* for other sizes) – appear to have the appropriately sized openings for productive sieving.

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