Estimating Population Sizes Using Mark-Recapture Techniques

Estimating the size or density of a population is a fundamental problem in ecology. When all individuals of a species can be counted directly, determining the abundance is straight-forward. In most cases, however, a direct count of all individuals is neither possible nor practical, and we must use indirect methods to estimate the full size of the population.

In this lab we will use mark-recapture data to estimate the size of the painted turtle population in the Zuck Arboretum ponds. In addition to estimating the size of the population in the Arboretum, we will also determine which subspecies of painted turtle is found here in Madison.

Painted Turtles

The painted turtle (*Chrysemys picta*) is a small turtle (11-15 cm) found in most of the United States and southern Canada. Adults typically have yellow or red markings on the sides of the carapace, yellow stripes on the side of the head, and red or yellow marks or stripes on the legs and tail. Painted turtles are found in a wide range of aquatic habitats, from lakes and ponds to streams and wetlands, though they are usually found in shallow, weedy, slow-moving waters. They are primarily vegetarian, but will also eat small crustaceans or mollusks.

Distribution of *Chrysemys picta* in the United States (From Ernst & Barbour 1972).

There are four different subspecies of painted turtles. The Eastern painted turtle (*Chrysemys picta picta*) and the Midland painted turtle (*Chrysemys picta marginata*) are both found in New Jersey, and they hybridize in places where their ranges overlap. The subspecies can be distinguished by the pattern of scutes on the carapace (top shell) and markings on the plastron (bottom shell). Scutes are the "plates" that fit together to make the shells.

Mark-Recapture Methods

One method for estimating the size of a population is to mark a known number of individuals and then resample the population to see what proportion of the newly caught animals are already marked. In addition to determining the size of the population, mark-recapture methods can be used to determine birth, death, and migration rates. The first ecological studies to use these methods were studies of fisheries in the late 19th century. Since then, many different techniques and analyses have been developed, each with its own assumptions and biases (Krebs 1999). Three of the more common methods are briefly described below; dozens of variations on these methods and other methods have been developed to handle different situations.

The *Petersen-Lincoln* method involves a single episode of marking and a single episode of recapturing. This method assumes that all individuals, marked and unmarked, have an equal probability of being recaptured (i.e. the sample is random), and that there are no births, deaths, or migrants during the study period (i.e. N is constant). This method is often used because it is the simplest method and the calculations are intuitive, but it tends to overestimate the true size of the population. The Petersen-Lincoln equation:

$$\hat{N} = \frac{CM}{R}$$

where $\hat{N}$ = estimated population size (a "hat" indicates that a number is an estimate)
M = number of individuals marked in the first sample
C = total number of individuals caught in the second sample
R = number of marked individuals recaptured in the second sample

The *Schnabel* method is an extension of the Petersen method that requires multiple episodes of marking and recapturing. It makes the same assumptions as the Petersen method, but it is easier to detect violations of the assumptions.

$$\hat{N} = \frac{\sum_t (C_t M_t)}{\sum_t R_t}$$

where the subscript t indicates the value for a parameter at each sampling time t. We will use the Schnabel method to estimate the size of the painted turtle population in the Zuck Arboretum. More details about calculating this value are in the "Calculations" section of the handout.

For populations that are changing in size because of birth, death, or migration, the *Jolly-Sebel* mark-recapture technique can be used. This method requires that each individual be given a unique mark and that there are multiple censuses, but it allows a population to be studied over long time periods. This method also assumes that every individual has the same probability of being caught, and that every marked individual has the same probability of surviving.

Consider the biological relevance of the assumptions that these different mark-recapture calculations make. Why are we using the Schnabel method for this study? What if we knew that we were more likely to recapture marked individuals? or females? or juveniles? What method would we likely use if we were studying insects? or fish? or zooplankton? How can we estimate population sizes of organisms that we can't mark?

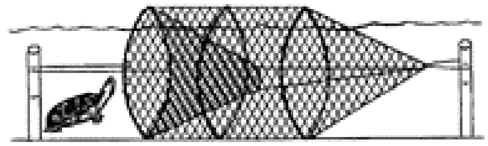
Confidence Intervals

The estimates of population size obtained from any of these methods are just that: estimates. In order to interpret these estimates, it is useful to know how reliable or accurate the estimate is. Statisticians use *confidence intervals* around an estimate to describe a range of values that is expected to contain the true value. For example, the 95% confidence interval represents a range of numbers around our estimate, and there is a 95% chance that the true size of the population is in that range.

The confidence intervals for an estimated population size calculated using the Schnabel technique are based on the Poisson distribution if there are fewer than 50 recaptures (we are unlikely to catch 50 turtles, let alone *recapture* 50!!), and can be found by looking them up in a table (see Table 2.1 at the end of this packet). It is worth spending some time looking at the table to see how the magnitude of the range of the confidence interval changes as the total number of organisms observed increases. A loose rule of thumb is that our estimate is not very reliable if $RM < 4\hat{N}$.

Field Methods

Trapping methods



Turtles can be caught by using long-handled dip nets or by setting up basking traps that float on the water and catch the turtles as they try to jump back in the water. We will capture turtles using hoop nets baited with sardines. The nets allow turtles to enter through the "funnel" end, but it is difficult for them to find the exit. (Think about the pros and cons of these different capture methods!)

Traps will be set up before our lab and will remain in place for a week of data collection. However, you may need to restake the net if you find it has collapsed, so make sure that you understand how the net works! You may also need to put in fresh bait (tinned sardines or catfood).

There will be a net in each of the ponds. Both nets will be checked daily for several days, so each of you will go out twice during the week to check the nets. Equipment and data sheets will be stored in the shed, along with all the other equipment needed to make measurements.

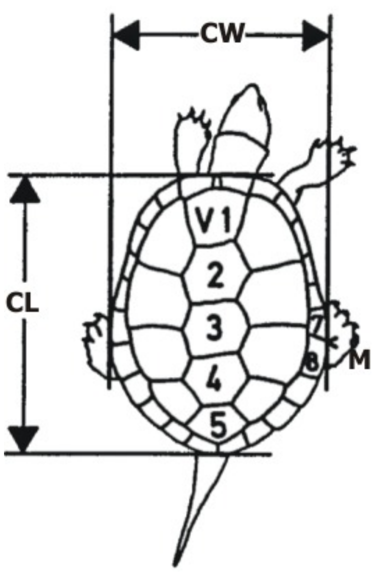
Handling the captives

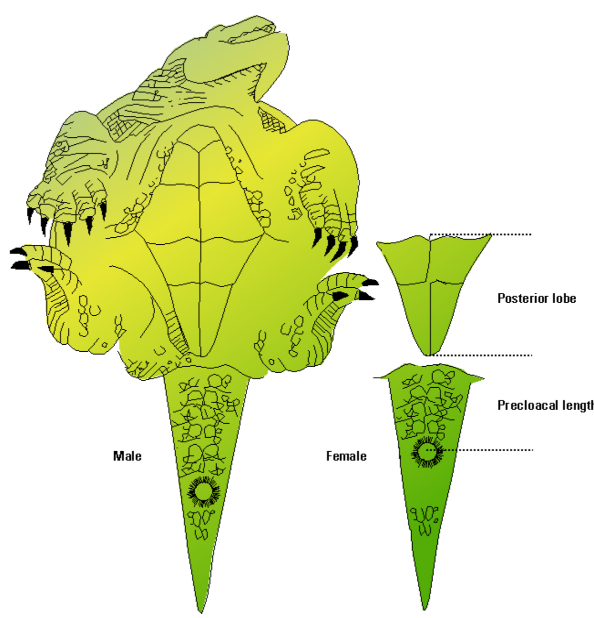
Be careful when handling live animals!! Take care not to injure the turtles, especially when removing them from the net. Although these turtles are small and generally harmless, they will bite fingers if given the opportunity!

<u>Measuring and weighing</u>

Weigh each turtle by placing it in a mesh bag and using a pesola scale. Be sure to tare the scale or weigh the mesh bag before weighing the animal!!

Using a measure tape or calipers, measure carapace width and length, precloacal length (an estimator of penis size, so useful in distinguishing sexes), and length of third right foreclaw (also useful for sexing animals). Carapace width (CW) and length (CL) are measured at the widest part of the carapace. Precloacal length (PL) is the distance between the margin of the plastron and the middle of the cloaca.





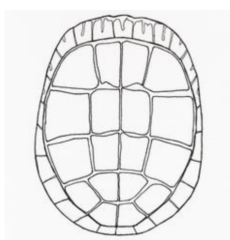
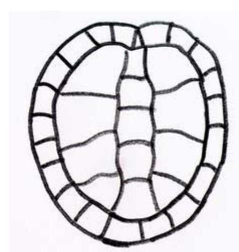
Sexing

Adult males and females can be distinguished by their size, overall shell shape, length of claws, and length of tail. It can be difficult or impossible to sex juveniles, so we will have 3 categories: male, female, and juvenile.

	Females	**Males**
size	larger	smaller
carapace shape	slight arch (accommodates eggs)	flatter
tail	thin, short tail	thicker, longer tail
anal opening	under rear margin of carapace	posterior to rear margin of carapace
foreclaws	shorter	longer, often white-tipped

Identifying subspecies

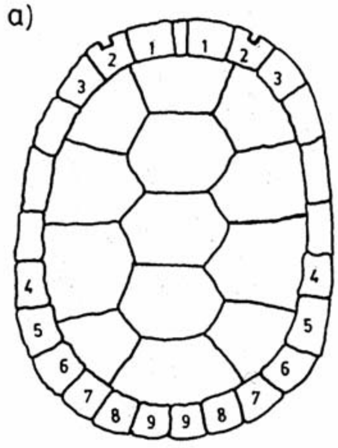
Two painted turtle subspecies are found in New Jersey. They have slight differences in shell morphology and color. Hybrids between the two subspecies might have intermediate characteristics. For each turtle caught, note the pattern of scutes on the back and if there are any marks on the plastron.

	Chrysemys picta picta **Eastern painted turtle**	*Chrysemys picta marginata* **Midland painted turtle**
plastron marks	solid yellow (or with small spot)	dark blotch covering most scutes
carapace scutes	in straight row across back 	alternate across back 
other marks	two yellow spots on side of head	similar to Eastern Painted Turtle

Marking

We will permanently mark turtles by making notches on the margin of the carapace with a clipper or file. Some turtles in the ponds have been permanently marked by Joe DiComo '09, who studied this population over the summer, and we will mark each turtle we catch if it is not already marked. Notches can be made with nail clippers or a file.

a)



Our marking scheme assigns a letter to each marginal scute, starting with A in the top right and moving clockwise around the shell (the "keystone" scute behind the head is unlettered). The twelve scutes on the right are A-L, and the twelve scutes on the left are M-X. For example, the diagram to the right shows a turtle marked "BW," because it has two notches. A list of marks already used will be left with the data sheets. Please update this list as you mark turtles!!

Calculations

We will use the Schnabel method for estimating population size. Again, this method assumes that the population is closed (N is constant, with no births, deaths, or migrants) and that every individual has an equal chance of being caught and recaught.

$$\hat{N} = \frac{\sum_t (C_t M_t)}{\sum_t R_t}$$

To make the calculations, it is helpful to set up a table listing relevant values for each sampling date. The table below shows an example of a frog population sampled over 5 days (example from Turner 1950 as in Krebs 1999). Make sure you understand how each column relates to the others.

date, *t*	**number caught (C_t)**	**number recaptures (R_t)**	**number *newly* marked**	**number marked at large (M_t)**
1	32	0	32	0
2	54	18	36	32
3	37	31	6	68
4	60	47	13	74
5	41	36	5	87

$$\sum_t C_t M_t = (32)(0) + (54)(32) + (37)(68) + (60)(74) + (41)(87)$$
$$= 0 + 1728 + 2516 + 4440 + 3567 = 12{,}251$$

$$\sum_t R_t = 0 + 18 + 31 + 47 + 36 = 132$$

$$\hat{N} = \frac{\sum_t (C_t M_t)}{\sum_t R_t} = \frac{12{,}251}{132} = 92.8 \text{ frogs}$$

To find the 95% confidence interval for this estimate, we need to find the 95% confidence limits on the number of recaptured animals (because it is uncertainty about whether the proportion of recaptured individuals in our total catch is proportional to the size of the entire population that makes our estimate uncertain). We look up the total number of recaptured organisms observed ($\sum_t R_t$ from our data table, x in Table 2.1) in Table 2.1 to find the 95% confidence limits on $\sum_t R_t$. We then recalculate our estimated population size using these upper and lower limits (warning: the upper limit on $\sum_t R_t$ will give us the lower limit on $\hat{N}$, since $\sum_t R_t$ is in the denominator). In this example we recaptured 132 frogs, and we find that the lower and upper limits are determined using the normal approximation formula given at the bottom of Table 2.1. If $\sum_t R_t < 100$ this would be much easier, because we could just look up the upper and lower limits on $\sum_t R_t$ directly!

$$\text{lower limit on } \sum_t R_t = x - 0.94 - 1.96\sqrt{x - 0.02} = 132 - 0.94 - 1.96\sqrt{132 - 0.02}$$
$$= 131.06 - 1.96(11.49) = 108.54$$

$$\text{upper 95\% confidence limit on } \hat{N} = \frac{\sum_t (C_t M_t)}{\sum_t R_t} = \frac{12{,}251}{108.54} = 112.87$$

$$\text{upper limit on } \sum_t R_t = x + 1.94 + 1.96\sqrt{x + 0.98} = 132 + 1.94 + 1.96\sqrt{132 + 0.98}$$
$$= 133.94 + 1.96(11.53) = 156.54$$

$$\text{lower 95\% confidence limit } \hat{N} = \frac{\sum_t (C_t M_t)}{\sum_t R_t} = \frac{12{,}251}{156.54} = 78.26$$

This produces 95% confidence limits of 78.26 and 112.87 frogs. So we are 95% sure that the true number of frogs in this population is between 78 and 113 frogs. Note that these confidence intervals are not symmetrical around our estimated population size – that is, 92.8 is not half-way between 78.26 and 112.87.

References

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Images from:

de Solla, S.R., C.A. Bishop, G. Van Der Kraak, R.J. Brooks. 1998. Impact of organochlorine contamination on levels of sex hormones and external morphology of common snapping turtles (*Chelydra serpentina serpentina*) in Ontario, Canada. *Environmental Health Perspectives* 106: 253-260.

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Questions

1. Use the Schnabel method to estimate the size, with 95% confidence intervals, of the painted turtle population in the Zuck Arboretum (you may well find it much easier to set up an Excel spreadsheet to do these calculations!!).

date, *t*	**number caught (C_t)**	**number recaptures (R_t)**	**number *newly* marked**	**number marked at large (M_t)**

Calculations:

2. Explain the assumptions we made in order to estimate population size. Discuss the biological reasons why each assumption is/is not reasonable.

3. Since all real populations are open (with births, deaths, and migrations, so that N is not constant), why would you ever used closed population estimators to determine population size?