*Tested Studies of Laboratory Teaching*
*Proceedings of the Association for Biology Laboratory Education*
Volume 38, Article 1, 2017

# Estimating Fish Population Size using a Mark-Recapture Technique

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The purpose of this laboratory exercise is to estimate the size of a closed population in the natural environment. The exercise can be completed in two laboratory periods. During the first lab period, students label, bait, and set traps, then retrieve traps, mark fish at a local pond, tally data, and return fish safely to the pond. During the second lab period, students capture fish again, calculate an estimate of population size based on the proportion of recaptures they make, and evaluate the validity of their estimate. The exercise was designed as a guided inquiry activity for an intermediate level undergraduate field biology course. Students could then choose among several methods to develop their own course-based research project. The activity could be used in an Introductory Biology, Ecology, Field Biology, or Ichthyology course, as a methods exercise or as a guided inquiry activity preparing students to answer their own questions.

**Keywords**: population density; mark-recapture; quantitative skills; guided inquiry

## Introduction

This laboratory exercise was developed for an intermediate level undergraduate class, Introduction to Field Biology. The aim is for students to learn a technique for estimating the size of a closed population, then use the technique to conduct sampling to answer their own question. Possible questions are listed at the end of the student hand-out as conversation starters. Ultimately, students present a project proposal, collect and analyze data, then give a peer-reviewed formal oral presentation and submit a written manuscript.

The exercise could be used in an Introductory Biology, Ecology, or Ichthyology course, either as a methods exercise or as a guided inquiry activity preparing students to answer their own questions.

This activity requires approval from the local Institutional Animal Care and Use Committee and may require a scientific collecting permit from your state's wildlife agency. Depending on your state wildlife agency's scientific permit requirements, you may be able to conduct the activity with your personal fishing license, but this is unlikely. Allow approximately 6 weeks from the time the application is submitted until you receive your scientific collecting permit. Consult with your institutional animal care and use committee (IACUC) Chair to allow ample lead time for review of your animal protocol and to complete any training required by your Institutional Animal Care and Use Committee. Both the wildlife agency and the animal care and use committee will require an annual report regarding number of animals captured and number of animals killed, including deaths due to handling stress. Typically, if there is any mortality, fish die within 12 – 24 hrs from handling during tattooing. The instructor or graduate TA should visit the field location 24 hrs after the Day 1 activity to check for and count dead fish (if any).

The instructor or graduate assistants should do preliminary trapping in candidate ponds. Preliminary collections can very likely be conducted with only a personal fishing license. The most abundant species in the traps should be chosen as the target species. Trapping time can be varied, but longer trap times give fish longer to figure out how to escape. Short trap times of 1 – 1.5 hours not only fit well into a 3 hour laboratory period, you are likely to catch more fish.

The exercise requires 3 hours on Day 1 and 3 hours on Day 2 plus time for data analysis and reporting after the lab is complete. Day 1 and Day 2 do not need to be consecutive. They may be one to several weeks apart. Having students work in groups of 3 – 4 provides for good division of labor.

# Student Outline

## Purpose

The purpose of this laboratory exercise is to estimate the size of a closed population in the natural environment.

## Background

Mark-recapture methods provide reasonable estimates of total population size in closed populations. A population is considered closed if few or no deaths, births, or emigrations or immigrations occur during the study period. The approach is to capture and mark many individuals in a short period of time, allow time for marked animals to return to the population and mix back in, then capture as many individuals as possible during a second sampling. The proportion of marked individuals in the second sample is used to estimate population size. Formulae for calculating population size are shown in the Data Analysis section.

This method depends on several assumptions being met: First, the marking technique does not affect survival of the marked individual. Second, marked individuals mix thoroughly back into the general population when released. Third, the likelihood of capturing marked and unmarked individuals is equal. Finally, the amount of time required to capture individuals is small compared to the total time of the study.

The marking method should be chosen for its non-interference in growth, maintenance, and reproduction. The mark should be easy to detect, should last at least as long the proposed study period, and should be relatively inexpensive, quick, and easy to administer.

After marking, only healthy, unharmed animals should be released to re-mix with their native population.

Each capture method introduces its own bias toward catching more of a particular age class, size class or sex. Recognize that you may be estimating the size of a subset of the population, not the entire population. For instance, our minnow traps have funnels 2.5 cm in diameter. Fish much larger than 3 cm in dorso-ventral cross-section will not be captured. This particular sampling method allows us to sample juvenile bluegill (*Lepomis macrochirus*), but not adults.

In this lab, you will capture and mark fish in two ways: by fin-clip and by subcutaneous paint injection.

## Procedure

### *Record Species and Site Description*

On the data sheet, list the Latin name of the fish species you will be counting and marking.

Also write a site description, for instance, Concrete Pond or Middle Pond, the geographic coordinates of the water body, and the town, county, and state in which it is located.

### *Day 1 - Capturing and Marking*

<u>Trap Deployment</u>

1. Attach 8 feet of nylon string to a minnow trap clip. Tie a numbered label to the other end of the string.
2. Open the trap and add bait, ¼ c dry dog kibble, to one side of the trap. Close trap with the clip.
3. Lower each minnow trap into the water near the edge at a depth of a few cm until it's resting on the bottom. The trap should be completely submerged, but you should have enough string above the surface to wrap the string around a rock or a plant stem for easy retrieval.
4. Record the number for each trap and the time each trap is deployed on the data sheet.
5. Leave traps undisturbed for 1 hr (approximately).

## Trap Retrieval and Fish Marking

The entire class should decide what batch mark or marks to use.

Fish captured on the same day should have the same mark, called a batch mark because an entire batch of animals receives the same mark. If fish are marked by fin-clipping with sharp scissors, the same fin on the same side of the body must be clipped for all fish, e.g. upper lobe of the caudal fin or tip of right pectoral fin. If fish are marked by subcutaneous paint injection, the "tattoo" should be in the same location on all fish, for example: right side, dorsal surface, anterior to dorsal fin. Both methods can be used on the same day, such that some fish are fin-clipped and some are tattooed.

Your group must decide who will be the trap handler, the fish handlers, and the data scribe.

1. At the end of 1 hr, each group should prepare an anesthetic bucket and a recovery bucket. Both buckets should contain approximately 4 L of pond water. Add 400 ml Tricaine-S concentrated stock (1 g/L) to the anesthetic bucket and nothing to the recovery bucket. Both buckets must be aerated.
2. The trap handler should retrieve the group's traps one at a time.
3. The trap handler pulls up a trap and transfers it to the counting and marking station quickly to minimize stress to the fish. If there are no fish in a trap, refresh the bait and re-set the trap to continue fishing until all other traps are retrieved and any captured fish are marked. It there are no fish after the second deployment, the data scribe records the trap number and 0 for **number of fish** on the Day 1 datasheet.
4. At the counting and marking station, one fish handler with wet bare hands should gently remove the fish from the trap and place them in the anesthetic bucket. AVOID TRANSFERRING THE DOG FOOD because it is greasy and messy. Dump saturated dog food in a bucket and dispose of it elsewhere. Do not dump dogfood into the pond because it will attract fish away from traps.
5. Note the time fish were transferred to anesthetic. **Fish should not remain in anesthetic for more than 5 minutes.** Once a fish lists to one side and lies still, it is sufficiently anesthetized. The second fish handler removes the anesthetized fish using a minnow net and places it on a flat, moist surface for marking.
6. The fish can be marked by clipping a fin with sharp scissors or by subcutaneous injection of acrylic paint. Marked fish must be counted off for the data scribe to tally as each fish is placed into the recovery bucket.
7. Marking with acrylic paint:
    - Draw acrylic paint* into a 1 ml syringe without a needle.
    - Fill the barrel about half-full, wipe the syringe tip and attach a 26-gauge needle.
    - Hold the syringe upright and gently push the plunger until paint flows out, but not air bubbles. Wipe the needle tip gently with a Kim wipe or small rag.
    - To mark the fish, place it on a flat moist surface. With one hand, gently restrain the fish so it doesn't slide away. Inject the paint at a pre-determined position along the dorsal surface. Orient the needle with the opening on the top side. Insert the needle at a shallow angle under the back of a scale and just below the surface of the skin. Push the needle forward until about half of it is under the skin. Gently push the plunger as you slowly withdraw the needle. The paint will polymerize beneath the skin.
    - Gently place the fish into the recovery bucket.

    * Bright pink or bright orange paint is the easiest to see. Neon colors work best. Yellow is okay. Green, blue, red, and purple do not show up well.

8. Once fish are upright and swimming normally in the recovery bucket, they can be released back into the pond by gently submerging the bucket and tilting it so fish can swim out. Release the fish in a shaded or sheltered location to minimize the chance of heat stress and predation.

NOTE: Even if no one in the group has handled or anesthetized fish before, if you are careful, you should expect <10% mortality.

*Day 2 – Recapture*

## Trap Deployment

1. Bait each numbered trap with ¼ c dry dog kibble. Close trap with the clip.
2. Lower each minnow trap into the water until it's resting on the bottom. The trap should be completely submerged, but you should have enough string above the surface to wrap the string around a rock or a plant stem for easy retrieval.
3. Record the number for each trap and the time each trap is deployed on the data sheet.
4. Leave traps undisturbed for 1 hr (approximately).

## Trap Retrieval and Fish Counting

Your group must decide who will be the trap handler, the fish handlers, and the data scribe.

1. Each group should have a bucket of aerated pond water to count fish into.
2. At the end of 1 hr, the trap handler should retrieve the group's traps two at a time.
3. The trap handler pulls up the traps and transfers them to the counting station quickly to minimize stress to the fish. If there are no fish in a trap, the data scribe records the trap number and 0 for **Total** number of fish and Number of **marked** fish.
4. At the counting station, each fish handler (with wet bare hands) should gently remove fish from the trap and count them off for the data scribe to record as fish are transferred into an aerated bucket of pond water (no anesthetic). AVOID TRANSFERRING THE DOG FOOD because it is greasy and messy. Dump saturated dogfood into a bucket to be disposed of elsewhere.
5. The data scribe must keep track of total number of fish and number of marked fish for each trap.
6. Once all fish are tallied, they can be released back into the pond by gently submerging the bucket and tilting it so fish can swim out. Release the fish in a shaded or sheltered location to minimize the chance of heat stress and predation.

## Mark-Recapture Data Sheet

Species _____ (Latin name, correct format)

Site (descriptive name)_____

Geographic coordinates _____

Town, County, State _____

**Day 1: Marking**

Date _____

| Trap Number | Time Deployed | Time Collected | Time Elapsed (hrs) | Number of fish |
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| Total number of live fish marked and released | | | | ***a*** = |

Record recapture data on the next page.

**Day 2: Recapture**

Date _____

| Trap Number | Time Deployed | Time Collected | Time Elapsed (hrs) | **Total** Number of fish | Number of **marked** fish |
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| | | | Total | **n** = | **r** = |

A. N (population estimate) = _____

B. Ratio of sampling time:study period _____

C. Proportion of population captured & marked _____

D. How reliable is your estimate of population size?

## Data Analysis

A. Calculate the Petersen-Lincoln estimator of total population in a closed population:

$$\widehat{N} = \frac{an}{r}$$

$\widehat{N}$= estimate of the total number of individuals in the population
a = total number of individuals marked on Day 1
n = total number of individuals captured on Day 2 (both marked and unmarked)
r = number of marked individuals captured on Day 2 (i.e., the recaptures)
Use the values of a, n, and r recorded on your data sheets to estimate the total size of that population.

NOTE: If r < 10, use the formula $\widehat{N} = \frac{(a+1)n}{r+1}$ to obtain a less biased estimate.

B. Calculate the fraction of time spent catching animals

Calculate the total study period from the time first trap deployed on Day 1 to time last trap collected on Day 2. Example: First trap deployed at 9:30 am on Day 1, last trap collected at 10:45 am on Day 2. Total study period = 25 hr, 15 min = 25.25 hr.

Calculate the fraction of total study period spent collecting samples as the average time a trap was in the water divided by the total time from 1st deployment to last retrieval. Example: Average amount of time a trap was in the water was 1.3 hr. 1.3 hr/25.25 hr = 0.05 or 5%.

C. Calculate the proportion of population captured

Calculate the percentage of marked individuals in the total population as a/N. Example: 36 individuals were marked on Day 1. N (population estimate) = 185. a/N = 19.4%.

D. Evaluate the reliability of your estimate.

Criteria: Generally speaking, if the proportion of the population captured is <20%, the population estimate is accurate only to order of magnitude. If the proportion captured is at least 20%, the population size estimate is relatively more accurate.

## Cited References

Henderson PA 2003. Practical methods in ecology. Blackwell Publishing, Malden, MA.

Hill J and Grossman GD. 1987. Effects of subcutaneous marking on stream fishes. Copeia. 1987(2): 492-495.

### Possibilities for Additional Experiments

- Compare population size between ponds with different depths or bottom profiles or amount of shade.
- Compare population size between small water bodies with different fish densities or different types of predators

## Materials

- 12 – 14 minnow traps, preferably galvanized with 1 inch diameter funnel openings (can be purchased from sporting goods stores such as Cabela's, Bass Pro Shops, etc.)
- dog food for bait; dry kibble for large breeds is easiest to use
- nylon string – at least 140 feet (orange or bright pink)
- surveyor's tape (orange or bright pink) to make trap labels
- Sharpies for making trap labels
- Minnow nets (small mesh fish nets) – 1 per group
- 5 gallon buckets – 2 per group
- battery-powered aerators, tubing, and air stones – 2 per group
- folding camp table – 1 for every 2 groups
- portable chairs – 1 per group for marking
- portable awning for marking station (if lab occurs in hot weather)
- Tricaine-S (ethyl 3-aminobenzoate methanesulfonate, Western Chemical)
- acrylic paint (orange or bright pink)
- 1 ml syringes
- 26 gauge needles, 5/8"
- cloth rags to wipe marking surface
- surgical gloves and safety goggles – 1 set for each student who will handle anesthetized fish/buckets
- storage container with secure lid for transport of used Tricaine solution back to campus

Tricaine-S (CAS 886-86-2) was purchased in the 10 g size from Western Chemical, Ferndale, WA; www.wchemical.com.

## Notes for the Instructor

**Gentle fish handling is essential for this activity to be successful.** Students who touch fish MUST have wet hands to reduce the amount of protective mucus rubbed off the fish. Fish survival is much better if the mucus is not rubbed off by contact with dry hands or cloth. Anesthesia before marking is strongly recommended to reduce stress and increase survivorship of the marked fish. It is advisable for students who handle anesthetized fish and/or who come in contact with buckets containing Tricaine to use protective gloves and safety goggles. Students must be careful to hold the fish firmly, but without squashing them during the marking process. The flat, moist surface mentioned in the student hand-out can be the plastic table top or a plastic cutting board, as long as the surface is damp. Puddles of water make the fish too slippery, so a rag to wipe water over the surface before marking the fish is a good idea. If using the fin-clip, holding the fish within the minnow net and exposing only the fin to be clipped works well. Both the fin-clip and acrylic paint marking technique have a 99-100% survival rate, if fish are handled gently.

Because handling is so important, make sure to watch the fish handlers carefully during the trap retrieval and marking stage. Students want the fish to survive, but may not be used to handling something squirming and slimy. Make sure students who volunteer to be fish handlers know what's expected of them. Students commented that they would like to see an experienced person demonstrate the tattooing technique while narrating the process. If the instructor simply describes what to do, the first student is nervous because everyone else is watching him or her.

A portable camping table with a chair for the person marking fish provides a stable work surface and makes learning the marking technique easier. Or, you may be fortunate in having an appropriate field site with picnic tables and benches. Take a large, labelled plastic jug with a secure lid to transport used Tricaine solution from the buckets used for anesthesia back to your campus, where you should consult with your chemical safety officer about proper disposal.

Make sure students are aware of the general hazards of outdoor work in your area and in the season during which they are working. Give them strategies for managing those risks. Also point out any plant or animal hazards around the location where they will sample. For instance, show them how to identify poison ivy and point out any patches near the sampling locations. Show them how to identify any poisonous snakes they might encounter and make sure they know where the nearest emergency room is and how to contact the instructor or TA in case of an emergency.

## Cited References

Henderson PA. 2003. Practical methods in ecology. Blackwell Publishing, Malden, MA.

Hill J and Grossman GD. 1987. Effects of subcutaneous marking on stream fishes. Copeia. 1987(2):492-495.

## Acknowledgments

Many thanks to all of the BIOL 2397 students who have helped improve this laboratory exercise over the past 2 years.

## About the Author

Ann O. Cheek holds a BS in Biology from the College of William and Mary and a PhD in Zoology from Duke University. She has taught at public universities in Texas and Louisiana and at Kingston University in England. She

has been an Instructor at the University of Houston since 2012, where she teaches large courses in introductory biology during the academic year and a small course in field biology at the University of Houston's Coastal Center in the summer term. In 2014, she received the Butler Award for teaching excellence in the College of Natural Science and Mathematics and in 2016 she was selected as a namesake for UH's off-site orientation experience, Cub Camp. Namesakes are nominated by students, faculty, or staff in recognition of their positive impact on first year students.

# Appendix A

This work is conducted under scientific collecting permit number SPR-0516-126 issued to Professor Ann O. Cheek by the Texas Parks and Wildlife Department and under animal use protocol number 16-023 approved by the University of Houston Animal Care and Use Committee.

## Anesthesia Stock Solution

Add 1.6 g sodium bicarbonate (Sigma-Aldrich) to 400 ml deionized water.
Add 0.4 g Tricaine-S (ethyl 3-aminobenzoate methanesulfonate; CAS 886-8602; Western Chemical, Ferndale, WA; www.wchemical.com) to 400 ml of 2 g/L sodium bicarbonate buffer.
Stir until dissolved.
Store in the refrigerator in an opaque or foil-wrapped plastic bottle for up to 3 days.
Transport in a cooler on ice to the field site. Pour entire 400 ml into 4 L ambient temperature pond water immediately before use as anesthetic.
Use an empty, labelled plastic jug with a secure lid to transport used Tricaine solution back to your campus; consult with your chemical safety officer about appropriate disposal on campus.

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## Citing This Article

Cheek, A. O. 2017. Estimating Fish Population Size using a Mark-Recapture Technique. Article 1 In: McMahon K, editor. Tested studies for laboratory teaching. Volume 38. Proceedings of the 38th Conference of the Association for Biology Laboratory Education (ABLE). **http://www.ableweb.org/volumes/vol-38/?art=1**