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Making a Museum: Preservation of Vertebrate Specimens for Use in Biological Laboratories

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Many instructors find it difficult to access specimens needed for teaching biology laboratories. This laboratory is adapted from an undergraduate/graduate course offered at the University of Wisconsin Madison, called Introduction to Museum Studies in the Natural Sciences. The laboratory, which is taught through the University of Wisconsin-Madison Zoological Museum (UWZM), provides instructors and students the opportunity to learn and practice proper preservation techniques of vertebrate specimens. This laboratory has been divided into two parts: Part A. Fluid Preserved Specimens and Part B. Preparation of Small Mammal Study Skins. In practice, each lesson takes between two and three hours from the beginning of the demonstration to the end of the hands-on student portion of the laboratory. However, this particular laboratory has been adapted so that both parts can be completed in a three hour session. After preparation, the specimens are available as teaching resources.

Keywords: museum, fluid preservation, study skin, UWZM (University of Wisconsin Zoological Museum)

Introduction

Museum specimens are an integral part of many biology laboratories and instructors who have access to museums and natural history collections find them to be an invaluable resource. Also, vertebrate specimens may be brought to your laboratory or offered to you through various means. Knowing how to prepare salvageable specimens for long-term storage and to make them available for your laboratories will allow you and your students to see and learn about animals from the specimens themselves. Participants will be introduced to various preservation techniques: mounts, study skins, disarticulated and articulated skeletons, fluid preserved, and cleared-and-stained specimens. However, this laboratory focuses on preparation of study skins and fluid preservation techniques. Attendees will watch a demonstration on fluid fixation and preservation and then practice these techniques on actual specimens. Next, they will learn how to make a more technically difficult museum study skin. Finally, each participant will practice making a museum study skin from a euthanized laboratory mouse.

Student Outline

Objectives

- Receive a brief introduction to a variety of museum preparation and preservation procedures.
- Watch a demonstration showing fluid specimen fixation and preservation.
- Practice fluid fixation techniques.
- Watch a demonstration of study skin preparation (mammal or bird).
- Practice study skin preparation.

Introduction

Part A: Preparation of Fluid Preserved Specimens

Fixation- A fixative is intended to arrest decomposition and to set in place the gross morphological and histological features of the specimen. The latter is achieved by denaturing and cross-linking of proteins. The traditional fixative is 10% buffered formalin, but strong ethanol is now sometimes used. Buffered formalin (10%) is a histological fixative. It gives a "firm pickle" (which is desired), however it obscures colors and is toxic. Ethanol (95%) is not a histological fixative. It gives a brittle pickle (which is not desired), but it preserves DNA and is nontoxic.

Preservation- Long term storage requires an appropriate fluid medium. Specimens are often rinsed/soaked in water, or stepped through a series of baths from weak to the final storage concentration, prior to being placed in the storage fluid. Storage containers may be of glass, stainless steel (tanks for larger specimens), or plastic. If plastic is used, it must be tested to ensure it is inert to the storage fluid. Lids must also be carefully chosen to minimize evaporation. Polypropylene lids, with a shim of an incompressible polyethylene, plastic or foam, are best. Some collections still use old canning jars which require a rubber gasket. Buffered formalin (10%) is used for amphibian and fish larvae. Ethanol (70%) is the standard storage preservative for birds, mammals, fish, amphibians and reptiles. Ethanol (95%) is used for tissues (samples must be taken prior to fixation). Isopropanol (50%) was traditionally used for fish, but most fish collections have switched to 70% ethanol for long-term storage.

Table 1. Recommendations for fixation and storage (prepared by Greg Mayer for Zoology 405: Introduction to Museum Studies in the Natural Sciences)

	Fixation	Storage
Larval amphibians, fish	10% buffered formalin	10% buffered formalin
Adult amphibians, reptiles, birds and mammals	10% buffered formalin or 95% ethanol	70% ethanol
Adult fish	10% buffered formalin or 95% ethanol	70% ethanol or 50% isopropanol

Methods

Part A: Preparation of Fluid Preserved Specimens

Before Lab

A) Freeze all incoming specimens. These include salvaged specimens, euthanized laboratory specimens or specimens procured from pet stores or other means.
B) Set-out specimens of various preparation types including mounts, disarticulated and articulated skeletons, and cleared-and-stained specimens, if they are available. Previously prepared study skins, and fluid preserved specimens should most definitely be on display.
C) The morning of the laboratory, gather all specimens and lay them out to thaw. Should you need to thaw them quickly, make sure each individual is bagged tightly and use a warm water-bath.
D) Set-up each station (2 students per station). Each station will receive a cafeteria tray, labels, pens, ruler, Pesola scale, small beaker with 95% Ethanol, syringes, needles, paper towels, fixing trays, glass jars and polypropylene lids.

Demonstration

A) Show students the various types of preparation methods (if they are available), especially the different fluid preservatives (ethanol, formalin, glycerin, and cleared and stained).
B) Show students how to "sex" the animal.
C) With a ruler, demonstrate standard measurements (in millimeters). These measurements vary by class of animal.
D) Using the Pesola scale, determine the weight to the nearest ½ gram.
E) Fill-out the specimen tag with all relevant collection information (collector and field number, locality and date of collection, and sex) on the front and measurements on the back.

Fixation of the Specimen

A) Depending on the proposed use of the specimen, the fixative may differ. However, for safety, when doing this laboratory with students, 95% ethanol is used as the fixative.
B) Lay the specimen down on the tray.
C) Inject ethanol into the mouth until it comes out the vent and into the vent until it comes out the mouth. Inject Ethanol directly into large muscles or tissues.
D) Tie tag onto the specimen.
E) Put the specimen into a fixing tray or jar, submerged in the fixative for three days.
F) Move the specimen into the collection jar with 70% ethanol for long term storage.

Part B: Preparation of Small Mammal Study Skins

Before Lab

A) Set-out specimens of various preparation types (if available). These can include mounts, study skins, disarticulated and articulated skeletons, fluid preserved and cleared and stained specimens.
B) Freeze all incoming specimens. Laboratory mice from on-line vendors work well for laboratory purposes because all specimens are the same species and relatively the same size.
C) The morning of the laboratory, gather all specimens and lay them out to thaw. A medium sized mouse will thaw in 2 hours. Should you need to thaw them quickly, make sure each individual is bagged tightly and use a warm water-bath.
D) Remove each individual from the bag. Remove any debris, droppings and spot clean urine stains. Pat or air dry to be certain that the skin is well dried before preparation begins.
E) Set-up each station (pods of 4 work well). Each student will receive a cafeteria tray, labels, pens, ruler, scissors, scalpel, sawdust, wire (0.024 gauge for tail and 0.033 gauge for legs), cotton, forceps, white thread and needle, probe and a mouse. Shared equipment includes: Pesola scales and wire cutters.
F) Set-up the drying station with Styrofoam and cardboard pinning board, lamps with 60 watt bulbs, many pins (glass headed, insect) and a tooth brush.

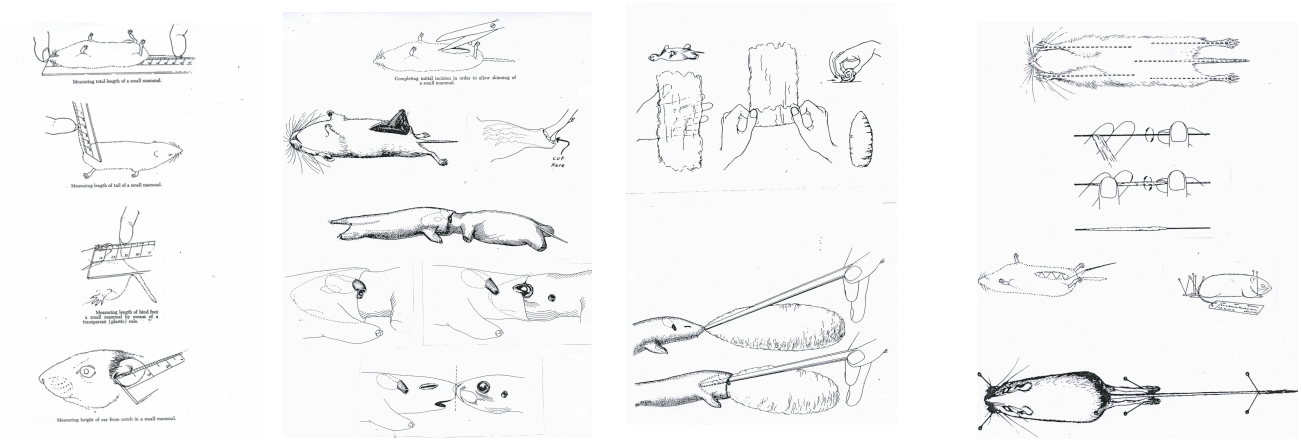
Demonstration

A) Show students the various types of preparation methods (if they are available).
B) Show students how to "sex" the mice (males have a scrotum and bigger gap between the genital openings; females have a closer gap between the genital openings). If possible, demonstrate the differences on a pair of well-developed mice.
C) With a ruler, demonstrate standard mammal measurements (in millimeters) in this order: total length, tail length, right hind foot length and right ear length. In the event that one of these elements is missing, make a note on the specimen tag.
    1. Total: tip of nose to tip of last tail vertebra with mouse lying on back on ruler.
    2. Tail: mouse on belly, hold tail at 45 degree angle, lay ruler on top of tail (with end of ruler where the tail meets the body) and measure to tip of last vertebra.
    3. Right hind foot: lay ruler on bottom of foot, measuring from heel to tip of longest claw (with nail).
    4. Right ear: put end of ruler into ear notch, gently lay ear up against ruler and measure to tip of ear.
D) Using the Pesola scale, determine the weight to the nearest ½ gram (hang mouse by back foot).

E) Fill-out the specimen tag with all relevant collection information (collector and field number, locality and date of collection, and sex) on the front and measurements on the back. Measurements should be recorded as follows: total-tail-right hind foot-right ear=weight (for example: 120-80-20-10=15g).

Preparation of the Mouse (see Fig. 1)

A) Lay the mouse on its back. Tent the belly skin (skin only) and put the sharp point of the scissors into the tent. Snip skin up toward the nose and snip down to right above the genital opening, making an incision that is large enough to get the leg through the hole.
B) Use fingers and nails to pull body away from skin. Be sure to use plenty of sawdust to absorb moisture and provide grip and to work the skin away from the body. Grip the mouse by the ankle and pull the knee through the opening. With the knee in between your fingers, use fingernails to work the skin down to around the ankle. Clear the skin and flesh all the way around and snip through the ankle just above the skin. Do the same on the other side.
C) Work around the back to the base of the back and tail. Pinch or cut off the skin attachments Hold the base of the tail firmly between forefinger and thumb nail and pull the tail out of the skin. [Note: Use particular care in the forefinger/nail grip and ease the tail vertebrae from the skin sheath. Tearing off of the tail skin is the commonest error in this procedure.] This should be carefully demonstrated by the instructor!
D) Work skin around the body to the forelimbs. Repeat steps used to release hind limbs.
E) Continue to work the skin up to the skull. Bring it up around the head to the base of the ears and snip the attachments with the scissors pressed against the skull.
F) Move down and hold scissors or a scalpel next to the skull to very carefully release attachments next to the eyes.
G) Make little snips with the scissors to release the nose and clip at the base of the lower incisors. Find the cartilaginous attachment at the end of the nose and clip straight down, being very careful near the nasals.
H) The mouse skin should be inside out. Remove all fat deposits from the skin. Use of sawdust is extremely helpful during this process. With sawdust too.
I) Use the body size of the mouse as a guide to fold (DO NOT ROLL) cotton. Lay the cotton down on the tray, and lay the forceps on top of the cotton, holding the very tip of the cotton with the tip of the forceps. Put the mouse "nose to nose" with the cotton batting/forceps and roll the skin onto the cotton. Adjust the skin, making sure the legs are pulled out. Remove forceps. Approximate where the base of the tail is and cut the cotton straight across (if you are unsure, make the cotton longer; you can always trim). Tuck cotton into the hind area of the skin.
J) Use the thicker (leg) wire to position the forelimbs first. Hold the front limb in between the first two fingers on your non-dominant hand. Shove the limb onto the wire over the wrist and into the palm. Clip the wire ¾ of the way down the body and repeat on the other side.
K) Again, with the thicker wire, hold the hind limb as above and shove the wire around the outside of the ankle and down into the footpad. Repeat on the other side. Clip wire ¾ of the way up the body.
L) To prepare the tail wire, get cotton damp. Hook a few strands of cotton across the top of the wire and spin the cotton very tightly with your fingers onto the wire. Continue to spin the wire, feeding threads of cotton until the new tail mimics the size of the tail removed with the mouse carcass. Feed the skin cautiously onto the tail wire. Clip the tail wire ¾ of the way up the body. This should be carefully demonstrated by the instructor!
M) Check the body shape and compare with the measurements.
N) To sew your specimen, start at the back with the needle on the inside of the body (no knot). Pull thread through, then make the knot around the skin to anchor it. Always go under and up, back and forth, under and up. Stay toward the outer edge of skin. When you get to the top, pull through at the end and clip very close to the body (again, no knot).
O) Tie your completed tag above the right ankle.
P) To pin the mouse, start with the front legs. Center the face between the front feet, which are palms down. Pin through each foot, making sure the feet are even and the body is straight back. Pull ankles in, close to the body, and put pins on outsides of back feet to anchor them. Center the tail and put pins on either side of the tail, crisscrossing above it at the base of the tail, in the middle of the tail and at the end of the tail. Pin ears back if necessary.
Q) Use the toothbrush to straighten fur.
R) Prepare the skull by cutting it off of the body. Remove the brain and eyeballs with a probe. Make a small tag with the field number and collectors initials on it, and attach it between the lower jaw and the mouth to make certain that the skull is always accurately matched with the study skin.



A B C D

Figure 1. Preparation of mammal skins, prepared by Paula Holahan for Zoology 405: Introduction to Museum Studies in the Natural Sciences. A. Depicts the procedures for proper measurements; B. Depicts the removal of the skin; C. Depicts the preparation and insertion of the cotton; D. Depicts the placement of the wires and the final position for pinning. Adapted from DeBlase A, Martin RE. 1981. A Manual of Mammalogy with Keys to Families of the World, Chapter 35, 2nd Edition.

Cited References

Pre-Laboratory and In-Class Handouts

Anthony HE. 1945. The Capture and Preservation of Small Mammals for Study. pp 21-42. The American Museum of Natural History: Science Guide No. 61. New York, NY.

Cogger HG. 1992. Reptiles and Amphibians of Australia. pp. 40-42. Comstock Cornell, Ithaca, NY.

DeBlase A, Martin RE. 1981. A Manual of Mammalogy with Keys to Families of the World, Chapter 35, 2nd Edition.

McDiarmid RW. 1994. Preparing amphibians as scientific specimens. pp. 289-297. In Heyer, WR, Donnelly, MA, McDiarmid RW, Hayek LC, Foster MS, eds., Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution Press, Washington, DC.

Materials

Equipment required for fluid fixation and mammal study skin preparation can vary by collection. These are the materials used at the UWZM for preparation.

For fluid fixation and preservation: a cafeteria tray upon which to work, rubber or nitrile gloves, a vertebrate specimen, labels (acid free or 100% cotton paper), pens with permanent ink (Pigma Pens), millimeter ruler for taking measurements, Pesola or spring scale for taking weight, 70% and 95% ethanol, syringes (lml, 5ml, or 10ml), needles (18-22 gauge), a fixing tray (a plastic container with a very tight fitting lid), paper towels, glass jars (8oz, 16oz, and 32oz) with a polypropylene lids with a polyvinyl liner.

Equipment required for preparation of mammal study skins: a cafeteria tray upon which to work, rubber or nitrile gloves (optional), vertebrate specimen (laboratory mouse), labels, pen with permanent ink (Pigma Pen), millimeter ruler, Pesola/spring scale, scissors, scalpel, sawdust, wire (.024 gauge for tail and .033 gauge for legs), wire cutter, cotton, forceps, sewing needle, white thread (sizes 40 and 8), pins (glass headed, insect), toothbrush, Styrofoam/cardboard pinning trays, lamps with 60 watt light bulbs, and a probe.

Notes for the Instructor

Be sure to check with university, local, state and federal agencies to make certain you have the proper permits and are following all guidelines to obtain and retain teaching specimens. Some suggested websites include:
Federal Migratory Birds:
https://www.fws.gov/birds/policies-and-regulations.php
International CITES: https://www.cites.org/

Properly preserved and stored museum specimens can be used for many years. To find basic and practical guidelines for storage of natural history collections, see the National Parks Service Conserve-O-Grams: https://www.nps.gov/museum/publications/conserveogram/cons_toc.html

Cited References

Anthony HE. 1945. The Capture and Preservation of Small Mammals for Study. pp 21-42. The American Museum of Natural History: Science Guide No. 61. New York, NY.

Cogger HG. 1992. Reptiles and Amphibians of Australia. pp. 40-42. Comstock Cornell, Ithaca, NY.

DeBlase A, Martin RE. 1981. A Manual of Mammalogy with Keys to Families of the World, Chapter 35, 2nd Edition.

Foster MS. 2012. Preparing reptiles as voucher specimens. pp. 95-125. In McDiarmid RW, Foster MS, Guyer C, Gibbons JW, Chernoff N, eds. Reptile Biodiversity: Standard Methods for Inventory and Monitoring. University of California Press, Berkeley.

Holahan PM. 2016. University of Wisconsin-Madison Course: Introduction to Museum Studies in the Natural Sciences. Manuscript.

Mayer GC. 2016. University of Wisconsin-Madison Course: Introduction to Museum Studies in the Natural Sciences. Manuscript.

McDiarmid RW. 1994. Preparing amphibians as scientific specimens. pp. 289-297. In Heyer, WR, Donnelly, MA, McDiarmid RW, Hayek LC, Foster MS, eds., Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution Press, Washington, DC.

Pisani GR 1973. A Guide to Preservation Techniques for Amphibians and Reptiles. Herpetological Circular 1, Society for the Study of Amphibians and Reptiles, Oxford, Ohio.

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About the Authors

Laura Monahan has worked at the University of Wisconsin-Madison Zoological Museum since 2006 and has served as the Curator of Collections since 2009.

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