TREC Herpetology Protocols

These protocols are adapted from Simmons (2002). Consult this source for detailed information about specific collection management topics. What follows includes the procedures followed at the TREC Natural History Museum.

Field Collecting

Collection methods for reptiles and amphibians are as varied as the animals themselves. Consult Simmons for suggestions on where and how to collect herps. All collection must be done according to the conditions listed on the collector's permit.

What to Collect and How Much – Collect only specimens that are needed to fulfill the museum's mission. Do not needlessly duplicate collections from this or other museums. Consult the records of other museums to determine what has already been collected from the area of interest. Don't collect specimens if it may harm the population. Do not collect more than can be processed in the time available. Do collect specimens that are insufficiently represented in collections. Juveniles and neonates are rare in collections, so efforts should be made to collect all life stages including eggs, larvae, neonates and immatures. An effort should also be made to collect a balance of both sexes in the adults. Ideally, larvae should be raised in the field, if possible, with one or more individuals preserved at each stage to yield a developmental series. Do not overlook bones, shed skins, parasites, hatched egg shells, or any other artifacts of the natural history of the animals that may have scientific value. Remember that photographs and sound recordings are also specimens and should be documented as accurately and in as much detail as physical specimens. Specimens collected dead should be refrigerated or kept on ice as soon as possible. Do not freeze them or place them in alcohol unless absolutely necessary. Ideally, they should be fixed in formalin immediately. However specimens are collected, they should be processed as soon as possible to ensure that they are in the best possible condition.

Field Notes – Field notes are the critical documentation that gives validity to scientific specimens. The Grinnell system for field notes is probably the most widely used, although it can be adapted to personal circumstances and preferences. See Herman (1989) for more information on using the Grinnell system. The system consists of three parts:

The field journal contains details of the trip, weather, rainfall, temperature, sun/moon observations, habitat type, lists of species observed. Each page is headed by date and locality.

The field catalog contains a list of specimens collected, their field numbers, and detailed collecting information, including place of capture, time of capture, temperature, weather conditions, habitat, microhabitat, and any other pertinent data. Any measurements or other data collected on each specimen are recorded here. Include time of death, time of fixation, precise methods of killing, precise method of fixing, source of chemicals and how prepared (example: if in field, stream water). Describe the specimen carefully, paying particular attention to details that will be lost with preservation, such as live weight and colors. Include references to any photographs, recordings, tissue samples or other related data. Use a single sequential set of field numbers throughout your career. Each page is headed by date and collecting locality, including GPS reading, if available.

Species accounts – these are detailed notes on specific species, with each page headed by the name of the species. Record observations on the behavior and ecology of animals that are not collected as well as those that are collected.

Localities – Accurate and precise locality data are essential. Record localities with both a written description and a GPS unit (if available). Do not write coordinates only – an error in a single digit can cause the data to be dramatically inaccurate. Record the datum setting of the GPS unit and the accuracy reading of each point recorded. Written descriptions should be clear and succinct, and should refer to features on standard maps.

Preservation of Specimens

The amount of care taken with the initial preservation of a specimen is arguably the most important factor in determining how long the specimen will remain useful in a collection. Follow proper procedures carefully, and make sure that chemicals used for fixation and preservation are fresh and of proper strength. Specimens may be preserved in the field or in the lab, depending on circumstances.

Processing kit
Hardening trays (polypropylene) with tight-fitting lids. Lock & Lock® containers are a good choice.
Paper towels to line the hardening trays.
10% buffered formalin.
deionized water
ethanol
Graduated cylinders
Notebook (including field catalog, if one is kept).
Pigma pen or other pen with non-acidic permanent ink
Containers for live specimens
Bottle forceps
Killing agents
Small forceps, probes, and dissecting needles for positioning specimens
Syringes and needles
Scissors, various
Scalpels
Scales
Metric ruler
Splash goggles
Nitrile gloves
Sewing needles
Tag string
Tags
Tag punch
Label paper
Specimen jars calipers
color chart
corks, wood wedges (for turtle mouths)
field tags
hand lens
nitrile gloves
references
sewing needles
spoon for larvae
tags

thread
tools for tissue samples
wire (for positioning)
camera
cheese cloth & plastic bags
large containers for tagged specimens
Procedure for processing specimens
1. Assemble all the equipment, your field catalog, photo equipment, specimens, and field notes.
2. Photograph specimens before they are euthanized.
3. Kill and process specimens individually, making a careful entry for each one in the field catalog.
4. Lay out specimens in the order they will be cataloged to facilitate tagging.
5. Clean up chemical spills and clean and put away all equipment.
6. Write your field journal.

Killing
Must be done humanely and in a manner that does not damage the specimen.
Reptiles – the preferred method is by injection of aqueous sodium pentobarbital (Nembutal®, Fatal Plus®) into the heart, but this must be done by a veterinarian. Other methods may be used (see Simmons, 2002). Ether is one option.
Amphibians – Immersion in chloretone is preferred, but it may not be available. Immersion in MS-222 solution or application of benzocaine based oral analgesic (Oragel®) to the head or venter are other options.

Fixation and Preservation
Specimens are fixed in 10% buffered formalin. The buffers should be monobasic sodium phosphate monohdyrate ($NaH_2PO_4H_2O$) and dibasic sodium phosphate anhydrate (Na_2HPO_4). If a prepared solution is not available, use distilled or deionized water to mix a 10% formalin solution. Always use eye protection and nitrile gloves when handling formalin. Formalin must be injected into specimens larger than about 5 inches snout-vent length. Specimens are injected with formalin before they are placed in the hardening tray. Don't inject too much formalin in any one spot. Small specimens, especially amphibians, don't usually need to be injected.

Reptiles. One pair of hemipenes should be everted in males before injecting or perfusing the body. Using a syringe of fixative or preservative, insert the needle on the left side of the base of the tail, and put your thumb over the right side of the tail and vent to prevent both hemipenes from everting. Apply gentle pressure until the hemipenis is everted, then tie a soft string or thread around the base of the hemipenis so that it remains extended.

Lizards should be injected in each limb, in the body cavity, and anteriorly into the gular region. Using a very sharp needle, make a series of perforations through the skin of the tail from base to tip on the ventral surface to allow the solution to penetrate, or wait 10-15 minutes and make a series of perforations on the dorsal and lateral surfaces of the tail.

Snakes should be injected ventrally at several points along the length of the body cavity. Very large specimens should be injected at several points along either side of the spine down the

full length of the body and tail. Make a series of perforations through the skin of the tail from the base to the tip on the ventral surface to allow the solution to penetrate.

Turtles. Extend the head and neck from the shell. Insert a small piece of soft wood, plastic, or cork in the mouth to keep the jaws open. Inject the preservative into the neck, limbs, tail and deep into the body cavity.
Large amphibians. Inject the limbs and body cavity.

Preparing the Hardening Tray
Specimens are next laid out in a flat-bottomed hardening tray. Line the bottom of the tray with a layer of paper towels and moisten them with the buffered 10% formalin solution. Smooth out the paper towels to remove the air bubbles and creases, providing a flat surface on which to lay the specimens. Do not use paper towels moistened only with water for this step, which may cause the specimens to contort and will dilute the formalin.

Positioning Specimens for Hardening
Specimens are positioned for hardening in a natural posture that allows (1) key features of the specimen to be accessed readily; (2) the maximum number of measurements to be obtained easily from the specimen; (3) the specimen to be inserted and removed from the container without tangling up with other specimens; and (4) packed for shipping without damage to digits or tails. The position should be close to a natural posture for the animal. Draw the limbs to the body, flexed in a natural position, with the fingers and toes extended and straightened. Spread amphibian toes apart to show the extent of the webbing. The tail of lizards can be curled up along one side if necessary to fit the final container. Snakes are coiled clockwise with the head to the outside. Turtles should have limbs, head and neck, and tail extended from the shell. The mouth should be held open with a piece of soft wood, plastic, or cork.

Hardening and Tagging
Once positioned in the hardening tray, specimens are covered with a layer of paper towels dampened with fixative, and a standing amount of buffered 10% formalin solution poured over them. Put the tray in a place where it will not be moved for several hours.

Small amphibians will harden in a few hours; larger specimens and most reptiles will require overnight.
Field tags should be affixed to the specimens after they are hardened. Tie tags on the specimens with a soft cotton thread. On animals with limbs, tie the field tag on the animal's left hind leg. If the left hind leg is not present, or is damaged, tie the tag on the animal's right hind leg, or around its waist. If the animal is so small that the tag will damage the leg, tie it around the animal's waist. For very tiny animals, place the tag and the animal together in a small vial without tying on the tag. Tags should be tied tight enough that they will not come off when the animal is handled, but not so tight as to cause distortion to the body. For snakes and other limbless animals, sew the tag through the neck, approximately one head length behind the constriction of the neck, but take care to sew below the vertebral column so the vertebrae are not damaged.
After the tags are affixed, transfer the specimens to a large container where they can reside submerged in the 10% buffered formalin solution for a week to ten days before further processing. Leaving the specimens in formalin solutions longer than a few days will cause darkening and loss of pattern in specimens as well as pose a risk of decalcification of bone.

Transfer of Specimens from Fixative to Preservative
After fixing, the specimens are preserved permanently in 70% undenatured alcohol. Denatured alcohol should not be used for museum specimens. Specimens should not be transferred directly from 10% formalin into 70% ethyl alcohol because that causes swelling and shrinking of tissues, which can damage the specimen. The recommended process increases the concentration of alcohol in steps of about 20% (20% > 40% > 60% > 70%) until a 70% concentration is achieved. This may not be feasible due to the cost of alcohol, so a transfer to 35% alcohol for a few days before placement in the final 70% solution is acceptable in most cases. Specimens not intended for long term preservation may not need to be staged into the final alcohol solution. Specimens should never be transferred from one type of alcohol to another (e.g. isopropyl to ethyl).

Cataloging Specimens
Cataloging is the process by which a specimen is assigned a *permanent reference number*. The catalog number associates the field data with the specimen, makes it possible to retrieve the specimen from the collection, and identifies the specimen for all subsequent uses in the collections. The *catalog number* is not assigned to a specimen until it has been through the complete accession process, because catalog numbers may not be assigned to specimens until the museum can demonstrate its ownership of them. Catalog numbers of deaccessioned or destroyed specimens should never be reassigned to other specimens.

Hand-written Catalogs and Electronic Data Storage
The hand written catalog is an *archival copy* of catalog data. The electronic database is a *backup copy*. A hand-written catalog made with archival materials will last for centuries, whereas, electronic media will not last nearly as long, nor depend on special equipment to read.
Hand-written Catalog Entries
The catalog is written on 100% cotton rag acid-free paper with archival ink (Pigma pen). Minor mistakes can be erased with a clean, high quality ink eraser. Larger errors should be crossed out with one line, the correction written above, then initialed by the cataloger.

The Cataloging Process

Once a collection has been accessioned, specimen identification should be confirmed, collecting locality information standardized, and the specimens assigned catalog numbers. The following should be included in the catalog record for each specimen.

Standard locality. Localities should be described so that they are easy to locate on maps, and should be specific enough to eliminate any ambiguities for subsequent users of the data. Georeferencing guidelines should be followed (see www.herpnet.org for links).

Coordinates. Include coordinates whenever possible. It should be indicated whether they were obtained with a GPS unit or calculated from a map.

The entry in standard format is written as follows:

State: Province, District, or Region: Other subdivision(s), as appropriate: Distance and direction to nearest map locality, coordinates, elevation

Example - PA: Erie Co: Presque Isle SP – Leo's Landing, 1.1 mi NE of park entrance.

Date. Enter dates as numerical day, the first three letters of the month, and the full numerical year as in 12 Feb 1809.

Scientific Name. No universally agreed upon lists of scientific names exists. The TREC Museum uses the ITIS website (www.itis.gov) for taxonomic names.

Collector. Collector(s) full name(s) should be recorded in the accession documents. Last names and initials may be used in the catalog record.

Field Number or Original Number. Record the field number for a specimen received directly from the collector, or the original museum catalog number for a specimen previously cataloged in another collection.

Preparation Type. The default for a herpetological collection is a whole adult specimen in an alcohol preservative. Note in the specimen record if the specimen is prepared as other than a standard preparation (e.g. a larva or dry skeleton, or a cleared and stained preparation).

Storage Medium. Note if the fluid preservative is something other than the standard preservative in use (e.g. larvae in formaldehyde), or if the specimen must be stored under special conditions.

Type Status. Note if the specimen is a holotype or paratype of a valid or synonymized taxon.

Developmental Stage of the Specimen. The default is a sexually mature adult. Note if the specimen is a juvenile, neonate, egg, larva, etc.

Accession Number.

Remarks. Record any references to any specimen documentation such as images, recordings, field notes or other permanent documentation. Note if the specimen was maintained in captivity before it was processed, if it was captive born, or captive raised. Record any other information as appropriate.

Specimen Database

Once all information has been recorded and gathered together, it is entered into the computer database. When a catalog number is assigned, it is input as "TREC- xxxxx."

Tagging Specimens

Tags are made from Tyvek® paper with a small hole punched at one end (about equidistant from both sides and one end). Size is 1 ¼" x ¼". The catalog number is written on one side, and "TREC" on the other. The tags are tied on with 100% mercerized thread without dye. Once a specimen has been assigned a catalog number, attach the numbered tag to the specimen.

1. tie the tag on the specimen's left hind leg, just below the constriction of the knee, firmly but not tight enough to damage the specimen. Ideally, both the collector's field tag and the museum tag should be placed in the same position on the specimen.
2. If the specimen's left hind leg is missing or damaged, tie the tag on the right hind leg, an arm, or around the specimen's waist, as appropriate.
3. For small snakes, lizards, and salamanders, tie the tag around the neck if there is sufficient constriction of the neck to keep the tag from coming off. Sew the tag on larger specimens or those lacking a suitable constriction of the neck. Sew through the neck below the vertebral column, or through the skin of the neck, with a thin needle.
4. For animals with limbs too small or fragile for leg tags, tie the tag around the animal's waist.
5. To associate numbered tags with specimens too small or too fragile for a waist tie (e.g. larvae), place the individual specimen and the tag (with about an inch of string attached) in a shell vial filled with the appropriate preservative. Position the specimen and the tag in the vial so that the tag can be read from the outside. Close the vial with a plug of polyester fiber. Avoid leaving bubbles below the polyester plug. Place the shell vial in a standard size jar (with closure) also filled with the appropriate fluid preservative.
6. Never remove field tags or previous field tags from a specimen unless the tags are deteriorating.

Preparation of Labels
Each tagged specimen in the collection is kept in a container (jar, vial, tank, bucket, box, etc) with a label bearing the specimen's catalog number, scientific name, and locality. The standard label is hand-printed on white 100% cotton acid-free stock with a Pigma pen.
Combining Specimens in Containers
As a rule, specimens should not be combined into single containers. If it is deemed appropriate to put more than one specimen in a container, only like-with-like should be combined. Do not mix specimens of different species, or from different localities, and do not overcrowd specimens. Maintain a ratio of at least twice the volume of liquid as specimens.

Container Labels
The standard label for a container should show the following information:
1. *Catalog number* of each specimen in the container. List multiple numbers in sequential order.
2. *Scientific name* of the species in the container.
3. *Family* that the genus and species is in.
4. *Locality* in standard format.
5. *Type status* of the specimen, if any.
6. *Preservative fluid* in the container, if other than the standard fluid preservative.
7. *Collector*
8. *Note* if the specimen should remain dry, or if the container is an empty jar marking the spot for a specimen in a tank.

Containers
Most specimens are stored in glass jars with screw-on polypropylene lids. Larger specimens may need to be stored in polypropylene storage containers (e.g. Lock'n'Lock®), or plastic

buckets with tight-fitting lids. Freeze-dried specimens may be stored in the appropriate dry room cabinet drawers.

Images

Images are designated with a TREC catalog number (e.g. TREC R- 00001) if no physical specimen was collected. The image then serves as the specimen, and is entered into the database for that group of organisms.

If photographs are taken of a collected specimen, they are designated with the catalog number of the specimen plus a lower case letter (e.g. TREC R- 00001a; TREC R- 000001b, etc.). These are recorded in the 'TREC Images' field in the database.

Digital image files are named with the catalog number of the specimen depicted, followed by the species (or higher taxon, if species not determined), e.g. "TREC R- 00001 Actinonaias ligamentina." Photographic prints and slides are labeled with the catalog number and the collection data normally written on a specimen label.

Images are stored as both digital files and prints. Digital images are kept in a designated folder, and prints are stored in archival albums labeled with the range of catalog numbers they contain (e.g. TREC R- 00001 – TREC R- 00120.

Video and audio recordings:

Video and audio recordings are designated in the same manner as photographic images, with each receiving a TREC catalog number if no physical specimen is collected, and an added letter designation for recordings of collected specimens.

Allocation of Specimens in the Collection

The TREC Museum stores herpetological specimens alphabetically by family, then by name (using the taxonomy of the ITIS website) on wire shelves in the Wet Storage Room. Specimens should be carefully placed in the proper tray every time they are returned.

Some points regarding specimen storage:

Always maintain at least twice the volume of fluid as specimens.

Never mix specimens from different dates or localities.

Use a container large enough that the specimens are not stressed, but small enough that space and preservatives are not wasted.

Containers should be filled to a level between the shoulder and the lid. Specimens must be completely covered by preservative fluid. Do not overfill – leave enough space between the fluid and the lid to allow for expansion due to temperature changes. A consistent fluid level makes it easier to determine if fluid is being lost.

If a specimen is partly protruding above the fluid level, cover the exposed part with cheesecloth that extends into the fluid to keep the specimen wet with preservative.

Check fluid levels regularly to be sure that preservative is not evaporating. Check lids for tightness.

If fluid is evaporating from a jar, wrap the threads with Teflon® plumber's tape and continue to monitor.

Larval amphibians should be stored in 10% buffered formalin. Work with them under a fume hood.

The storage environment must be monitored to provide optimum conditions for long-term preservation. This includes minimizing temperature fluctuations, light (especially UV radiation), and humidity.

References:

Simmons, J.E. 2002. Herpetological Collecting and Collections Management. Rev. ed. Society for the Study of Amphibians and Reptiles. Herpetological Circular no. 31.

Herman, S.G. 1989. The Naturalist's Field Journal. A Manual of Instruction Based on a System Established by Joseph Grinnell. Buteo Books, Vermillion, S.D. vii + 200 pp.