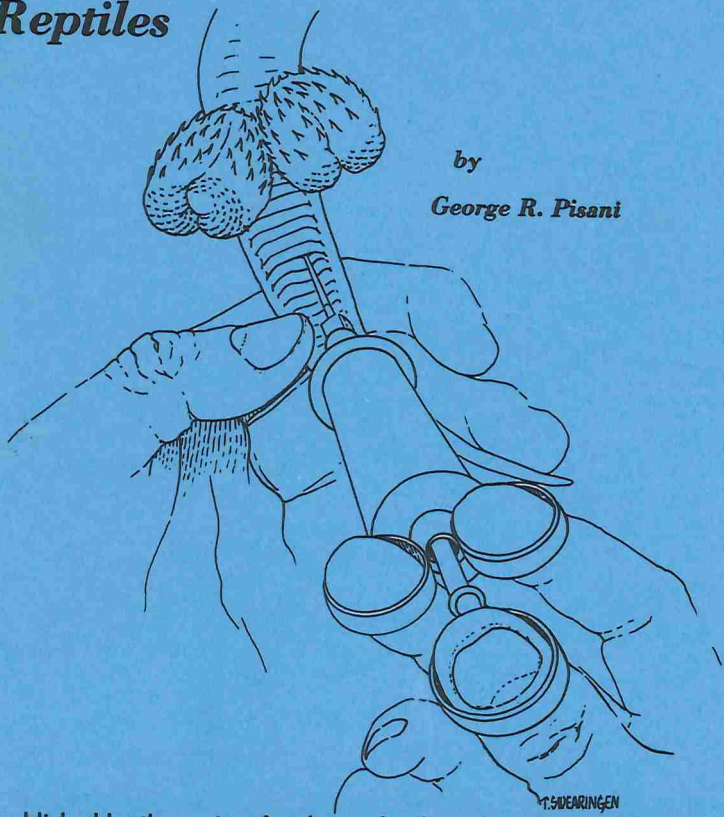
A Guide to Preservation Techniques for Amphibians and Reptiles



by

George R. Pisani

published by the society for the study of amphibians and reptiles

EDITOR'S NOTE

This is the first of a new series of Miscellaneous Publications entitled "Herpetological Circulars", designed to provide basic information for the amateur or beginning herpetologist. Museums, zoos, and laboratories will find it useful to distribute in response to inquiries concerning: current procedures used in preserving amphibians and reptiles; maintenance of collections; what information is recorded in field notes; how to ship specimens; color preservation. Brand names (and common names) of reagents, fixatives, paper and the address of the source of supply, are cited when applicable. A Spanish edition is available.

Single copies are available at US $1.00 each ($0.75 each for orders of 25 or more) including postage. Orders may be sent to Dr. Henri Seibert, Department of Zoology, Ohio University, Athens, Ohio 45701, U.S.A.

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A list of other Society publications, including those of The Ohio Herpetological Society, Facsimile Reprints in Herpetology, Herpetological Review, and the Catalogue of American Amphibians and Reptiles, will be sent on request by the Publications Secretary, Henri C. Seibert, Department of Zoology, Ohio University, Athens, Ohio 45701, U.S.A.

Membership in the Society includes subscription to the Circular series, Facsimile Reprint series, the Society's technical journal (Journal of Herpetology), and a newsletter (Herpetological Review). Currently, Regular dues are $10.00 ($8.00 for students), Sustaining $15.00, Contributing $20.00. Institutional subscriptions are $20.00. All inquiries about membership or subscriptions should be addressed to the Treasurer, Henri C. Seibert (address above).

A GUIDE TO PRESERVATION TECHNIQUES FOR AMPHIBIANS AND REPTILES

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INTRODUCTION

Over the years, several informative works describing the preservation of amphibians and reptiles have been published. Most of these have been intended for relatively limited distribution by the institutions or individuals publishing them. This fact, coupled with new laws pertaining to syringes and certain drugs used for killing specimens, warrants an additional treatment of the subject. This article is an attempt to combine a complete survey of current techniques with a page size that the individual collector can conveniently carry in the field.

In an age when so many wild species and areas of suitable habitat are at the threshold of extermination, it seems advisable at the outset to include a plea for conservation in this booklet. Current museum collections contain excellent samples of various North American species of reptiles and amphibians from certain areas within their ranges. In these instances, it is a needless waste to collect and preserve additional material when this will not add appreciably to our knowledge of these creatures. I am not referring to such collecting as may be associated with the compiling of a synoptic teaching collection by a school or to collection of specimens needed for a particular aspect of research, but rather to the capture and preservation of animals simply to amass a collection which may never be used for scientific or educational purposes. There are numerous geographic areas, including several in North America, in which the amphibians and reptiles are poorly known. Collections from these areas can add measurably to our herpetological knowledge. Persons wishing to learn of the desirability of specimens from particular areas should consult with herpetologists at nearby universities, museums, zoos, etc.

I would like to express my thanks to Woodrow W. Barber, Hobart M. Smith, William E. Duellman, Joseph T. Collins, Clarence J. McCoy, and George Iannarone for furnishing helpful material and/or advice and to Phyllis Shaffer, Judy Hamilton, Leanne Johnson, and Ginger Stiggins for typing assistance. Thanks are also expressed to Jaime Villa for preparing much of the "International Shipment" section.

FIELD NOTES

Specimens not accompanied by data identifying the collection locality are virtually useless to scientific investigators. The more data available for a specimen, the greater its value in research. Hence, keeping accurate, complete field notes is necessary. Many times, data felt to be trivial at the time of collection may prove to be quite useful when many observations are pooled. Field notes should be written in waterproof ink ("Pelikan" brand is preferred by many) using only one side of each page. Several brands of waterproof ink will "run" if alcohol is accidentally spilled on the page, hence care should be used in selecting ink. A worthwhile technique is to carry a small notebook for on-the-spot data taking. Then transfer these data into the permanent field notes as soon afterward as possible.

The following is a representative outline of data included in field notes (numbers refer to those in figure 1a):

1. Locality- Do not record locality with reference to business establishments. Use towns or mapped roadways; determine distance from an automobile odometer (if available), or estimate distance carefully from maps. It is not unusual for roads to be rerouted, renumbered or both. It is therefore advisable to refer to roads indicated in a good atlas to which future reference can be made. In the U. S., the American Highway Atlas (Gousha Co., Chicago) is suitable. If collecting in areas remote from roadways, locate the collecting site as accurately as possible from U. S. Geological Survey topographic maps. Collectors in foreign countries should try to obtain accurate, detailed maps of the areas in which they are working. Elevation of the locality should also be recorded whenever possible.

2. Date- Always write out the name of the month, or indicate month by a Roman numeral; 6-10-71 could refer either to June 10th or October 6th.

3. Name(s) of all collector(s) present.

4. Time of collecting.

fig. 1a

JTC 1971

Kentucky. Mc Creary Co. 7 mi W Cumberland Falls on Ky. Rt. 90 ←1

23 May 1971 ←2

8 → 4467 Pseudotriton ruber

3 → J.T. Collins ♂ G.R. Pisani – overall dark red with much dark mottling. mottling creates a purplish caste. Black flecks on chin and belly. Also black flecks on undersurface of all limbs. Found under deep wood near an abandoned sawmill at mid-day. Sex ?.

5 → Air temp 26°C, 21°C under log. ← 4

Kentucky: Pulaski Co. near Keno (P.O.) on Ky. Rt. 761, 1050'

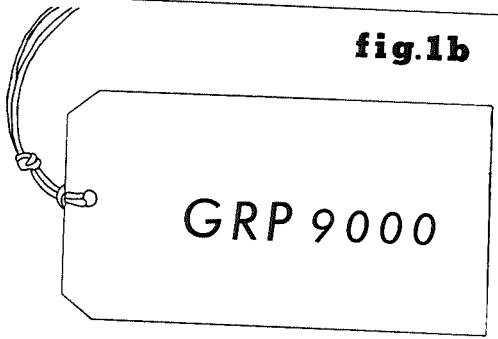
24 May 1971

6 → 4467 Storeria dekayi

J.T. Collins ♂ G.R. Pisani – overall color greyish-brown, double row of black spots flanking mid-dorsal grey stripe which runs from neck to tail. Belly pale with scattered black dots present on lateral edges of ventral scutes. Two black occipital spots. Found under flat

7 → limestone rock in wooded area at dusk. Sex ♂. Air temp. 21°C, 22°C under rock.

fig. 1b



5. Air temperature and other appropriate weather notes- It is often useful to note existing cloud cover and moisture conditions, as well as general weather conditions preceding the collection.

6. Species- List all species collected plus the number collected of each, followed by species which may be observed but not collected. Accurate color notes are a worthwhile inclusion, especially when collecting in regions having a poorly known herpetofauna. It is also worthwhile to take accurate color notes when an atypically colored individual of a well-known species is encountered.

7. Microhabitat of species collected and any significant behavior (courtship, defensive display, etc.) observed.

8. Field number- It is useful to carry a series of numbered field tags on collecting trips. These should be printed on heavy paper in permanent ink. Satisfactory tags can also be made with one of the commercially available label makers that imprint plastic tape. Thread can be sewn through the numbered tags, but the backing on the tape should not be removed. Avoid using colored thread, or thread made from synthetics such as nylon, which may be destroyed by preservatives. White, cotton carpet thread is suitable for tagging. After threading the tag, tie a small knot in the string as shown in figure 1b. Each specimen should be assigned its own number, which greatly simplifies the task of keeping specimens and localities associated. Testes, stomach contents, photos, tape recordings, etc. are assigned the same number to increase efficiency in future analyses. Field tags should be securely tied (with a square knot) to specimens as illustrated in Plates 1 and 2. Lizards possessing femoral pores should be tagged by knotting the string below the knee; this avoids covering the pores with string. Tags in the field series should be numbered independently from the catalog series discussed in a later section.

Of the above data, numbers 1 (locality), 2 (date), 3 (name(s) of collector(s), and 8 (field number) represent the minimum data which should be recorded. If specimens are donated to an institution, the field notes should be donated with them. Do not include field notes in the same container used to hold specimens.

KILLING OF SPECIMENS

It is essential that live herpetological specimens be killed in such a manner as to leave the muscles in a relaxed state. Following this, they can be fixed, or hardened, in standardized positions which enables researchers to examine them conveniently and most accurately (refer to Plates 1 and 2). Many books recommend that reptiles be killed by hypodermic injection of aqueous sodium pentobarbital (Nembutal) into the heart. This technique is indeed excellent, but the reader should be aware that Nembutal is not a generally available drug, its possession being closely regulated by the Federal Bureau of Narcotics and Dangerous Drugs. It is possible for qualified persons to obtain a permit to purchase Nembutal, but the application procedure is best begun several months in advance of anticipated need. For additional details concerning the permit, the reader is urged to consult representatives of the above-mentioned Bureau at the Federal centers in most large cities. State and local regulations should also be checked. Commercial Nembutal is sold at a concentration of 50 mg/cc. The form of Nembutal sold as a syrupy elixer should be avoided. Commercial Nembutal may be used directly for larger specimens (over 5 pounds body weight), and diluted 1:5 with water for smaller reptiles; for very small specimens such as Typhlops or small Scincella it is possible to dilute to 1:10 and retain effectiveness. Nembutal diluted 1:10 can also be used on larger specimens, but death will be delayed. One cc (used commercial strength) injected into the heart is generally sufficient to quickly kill an animal of the bulk (volume) of a 3 foot timber rattlesnake (Crotalus horridus). Position of the heart in snakes can often be judged by closely watching the ventral plates on the anterior 1/3 of the body to detect heartbeat. Injection anywhere into the anterior 1/3 of the body cavity is also effective, but death is not as rapid as from heart injection. Other reptiles can be killed by injection into the heart region. Do not attempt to inject specimens which are so small or thin as to be heavily damaged by the needles at hand.

A number of other effective killing means are available. Turtles may be chloroformed if care is taken not to allow them to stiffen. Confining the turtle with a chloroform moistened rag or cotton wad in a closed container for 15-30 minutes (Cook, 1965) should suffice. The use of chloroform on other reptiles is definitely not recommended, as severe contortion usually results. Trichloroethylene or ether may be substituted for chloroform with good results, and can be used on most reptiles. Most specimens can be killed by confinement with either trichloroethylene or ether for 5 minutes beyond the time the animal loses the ability to right itself when turned over. These liquids are available to the public from either biological supply houses or certain drugstores. Their use may be superior to Nembutal when working with small, fragile animals like some tropical geckos. Caution should be observed with ether, as it is highly flammable and can, under certain storage conditions, explode. Read labels carefully.

All amphibians and a number of smaller reptiles (e.g. small, tropical geckos) are easily killed by immersing them in a solution of Chloretone (hydrous chlorobutanol). A stock supply is commonly prepared as a saturated solution of Chloretone in 95% ethanol. This stock solution may be conveniently carried in a small vial; 2 cc of it added to a pint of water is effective. The solution should be kept tightly covered when not in use, and can be used over and over; the diluted solution's strength will diminish with use.

Various other means are suitable for killing reptiles and amphibians. Securing the animal(s) in a cloth sack and immersing the sack in warm (110°-120°F; 43-47°C) water is effective, but specimens should be removed immediately after death. Specimens may also be immersed in alcohol (15-25% for amphibians; 50-60% for reptiles). Though the method is not recommended, bags containing reptiles may also be left exposed to direct sunlight until death from overheating occurs. Great care must be used however, as dehydration and accompanying contortion can happen quickly; amphibians should never be killed in this way. Both procaine hydrochloride (Livezey, 1958) and succinylcholine chloride (Anectine) (Lambert, 1967) have been used effectively as killing agents; however, their availability is usually restricted like that of Nembutal.

Recent drug laws have greatly increased the difficulty of obtaining syringes for preserving purposes. State laws may also vary in the regulation of the above-mentioned chemicals. It is often possible to obtain necessary supplies through institutions, particularly in return for depositing desired specimens.

FIXING

The purpose of fixation is to preserve the actual morphological state and color of the specimen, and to prepare the tissues for microscopic examination. Hence, the fixative should kill tissue quickly; penetrate it uniformly and rapidly; prevent postmortem decomposition; not distort the tissue; and should prepare the tissue for staining. No single fixative will do all of these things, so various compromises must be made.

The most widely accepted and suitable general fixatives for field use are:

1) Formalin ("Formol" or "Formalina" in Spanish; "das Formaldehyd" in German)- Sold commercially as a solution of approximately 40 percent formaldehyde gas in water, formalin is the most widely used field fixative. For purposes of dilution, commercial formalin is usually considered as 100%, and can be used in 10% strength (1 part formalin: 9 parts water) for fixation. Formalin may be buffered (which helps to reduce discoloration of specimens) by mixing 1 tablespoon of baking soda or borax with each pint of 10% formalin. Generally sold as a liquid (often in drugstores), it is also available as a solid polymer (paraformaldehyde), which is convenient for saving weight and space in transport. Huheey (1963) recommends sealing 16 grams of paraformaldehyde and 4 grams of anhydrous sodium carbonate in packets for field transport; 1 packet added to 400 ml (about 1/2 quart) of water makes a 10% solution of buffered formalin. Premixed, buffered paraformaldehyde powder is available from Carolina Biological Supply House. Paraformaldehyde alone can be obtained from Eastman Organic Chemicals, Rochester, New York. Formalin, while an excellent general fixative, is highly irritating to the user's skin and (as a vapor) to mucous membranes. It is not uncommon for users to develop strong allergies

to formalin. Also, formalin has a tendency to cause swelling of several types of tissue, rendering them unsuitable for some histological purposes.

2) FAA (formalin-alcohol-acetic acid)- Prepared by mixing 10 parts commercial formalin, 50 parts of 95% alcohol (ethyl or isopropyl), 40 parts water and 2 parts glacial acetic acid. FAA penetrates tissue far better than formalin alone, and has less tendency to cause cell distortion. The rapid tissue penetration can also be an aid to preserving valuable specimens found dead and, perhaps, partially decomposed. The primary disadvantages of FAA are: the need to mix several components; and, the necessary alcohol and acetic acid may not be available in certain localities. FAA is not available in powder form, but can be premixed without the water to reduce volume in transport; water may be added later. If FAA is to be used extensively in hot regions, it is recommended that the acetic acid be added just prior to actual use, as it quickly evaporates from the solution; containers may be cooled by wrapping them in wet rags and shading them to retard evaporation of acetic acid.

3) Alcohol- If neither formalin nor FAA are available, alcohol may be used as a fixative. Cook (1965) recommends ethanol (95% for reptiles; 70% for amphibians) or isopropanol to fix in the absence of other solutions, but the latter is not desirable.

4) Special- A large number of other fixatives exist, each being useful for different types of tissues, and studies. Bouin's solution (75 parts saturated aqueous picric acid, 25 parts commercial formalin, 5 parts glacial acetic acid) is especially useful for field preservation of testes to be used in spermatogenesis studies. Testes may be placed in vials of Bouin's and safely kept there for long periods of time without distortion of cells; the remainder of the specimen may be fixed with FAA or formalin. For a complete discussion of special fixatives, the reader is referred to Guyer (1961) and similar texts.

5) Miscellaneous- If a valuable specimen must be saved and no other solutions are available, a number of emergency measures are possible. The specimen may be frozen or packed in strong brine until preservative can be obtained. Liquor is generally not a suitable source of alcohol, as 110 proof liquor is only 55% ethanol. However, strong tequila (about 160 proof) may be useful; rubbing alcohol can also be used. These, however, are only desperation measures and it is usually more beneficial to get the specimen into a proper fixative (hospitals, local schools, etc. are suggested as possible sources).

It is always preferable to introduce fixative into the body cavity, as specimens (particularly reptiles and larger amphibians) can decompose internally if simply placed in fixative. Enough fixative should be injected to fill, but not distend, the animal. Care should also be taken not to damage the femoral pores of many lizards by puncturing them with the needle. The neck of turtles should be completely extended and the mouth held open with wood, cork, or tightly wadded paper prior to fixation. Excellent neck extension can be obtained by hooking the dead turtle's upper jaw over a nail or broken branch and letting the animal's hanging weight pull the neck out straight prior to injecting it. The upper jaw can also be hooked over a paper clip placed over the edge of the fixing tray, and the neck then drawn out. One hemipenis of male lizards and snakes should be partially everted with thumb pressure on the base of the tail, followed by injection to completely evert it as indicated on the front cover. The hemipenis should not be permitted to remain incompletely everted; thread may be tied around the base of the fully everted hemipenis to help retain fluid within it. It is also an acceptable practice to evert the hemipenis by injection of fixative alone. Typical sites for injection of preservative are starred in Plate 2. Tails of lizards and snakes should be slit lengthwise, being very careful not to break the tail off; sharp instruments are a "must." Large amounts of fixative can be conveniently handled in the injection apparatus designed by Jackson (1971), although the author has never felt at a disadvantage using larger syringes. If no injection apparatus is available, the specimen should be deeply slit in several places ventrally and placed belly-up in fixative. Spread the sides of the slits to admit fixative more easily. Avoid cutting the anal plates of snakes and lizards and femoral pores of lizards.

Once the animal is injected or slit, it is most conveniently fixed by placing it (after proper positioning) between pieces of white paper toweling moistened liberally with fixative. This can be done in shallow, covered plastic or rustproof metal pans. Surgical instrument pans with sliding metal covers are very handy for this. Avoid colored towels, as the colors dissolve in the fixative and stain the specimen.

Preferred positions for fixing and sites for field tag attachment are illustrated in Plates 1 and 2. Amphisbaenids and caecilians should be fixed in the same position as snakes; it is useful to fix these with the mouth open, as this greatly facilitates examination of oral characters later on. Lizards with long tails should be fixed with the tails bent as shown. Frogs and toads may be positioned with the sole of the foot down (Duellman, 1962). However, because this position obscures many hind limb and anal characters, others feel that anurans are best fixed with the hind limbs in the position shown in Plate 1c. Toes and fingers should always be straight and spread apart. Small amphibians need not be injected or slit prior to positioning, as the fixative will penetrate to the body cavity quite easily. Small amphibians and lizards may have the field tag tied around the body just anterior to the pelvic region.

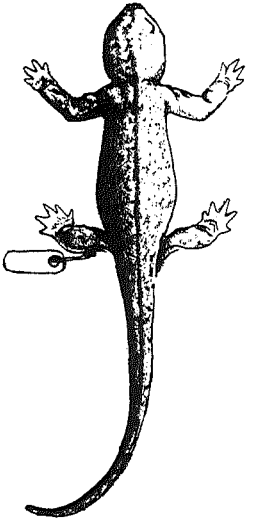
Amphibian eggs and larvae are best fixed and stored by dropping them directly into jars of 10% formalin; preserve entire egg clutches whenever possible. Many amphibians attach their eggs to leaves, twigs, etc. Whenever it is practical, these items should be preserved with the eggs in situ, as the latter are often severely damaged by attempts to disengage them. Change the formalin on eggs and larvae after about 12 hours. Reptile eggs should be measured (length and width, in millimeters), then injected.

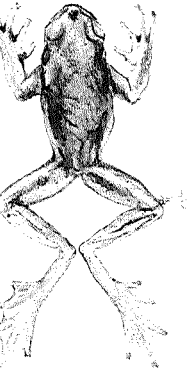
All specimens should be allowed to remain in fixative for 24 hours.

Very Large Specimens (too large to be conveniently stored entire in liquid)

1. Snakes- Record the snout to vent and tail lengths (in mm). Then skin by making a long ventral incision to the side of the mid-line; leave the head and tail attached to the skin, severing these from the carcass (avoid cutting the anal plate), and then inject head and tail (evert hemipenis if male) with fixative. With boids, sever the hind part just

Plate 1. Amphibians





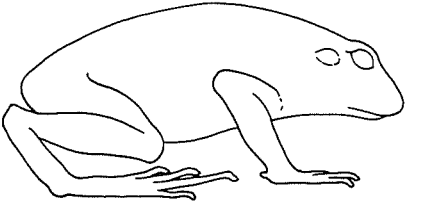
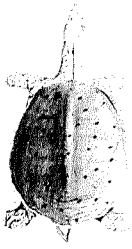
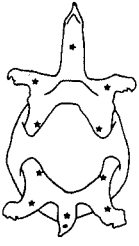
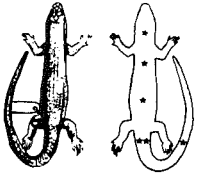
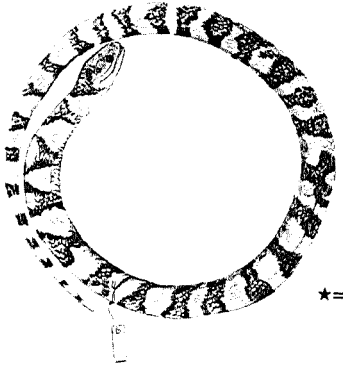


Plate 2. Reptiles









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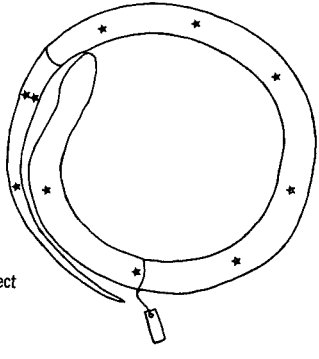

ahead of the bony, vestigial pelvic elements. The skin may now be preserved by covering the flesh side with cloth or absorbent paper, rolling loosely and immersing in fixative, or by rubbing with borax or arsenical soap, rolling and drying. In this latter instance, it is best to preserve the head and tail separately in liquid. If the specimen is a male, a testis should also be preserved. Reproductive condition of females should be noted (i.e.- number of ova present, size of the largest ovum, etc.). Embryos, especially those of poorly known species should be preserved in liquid; it is preferrable to do this by preserving the entire oviduct rather than by removing embryos.

2. Turtles- Avoid cutting the shell. It is preferable to cut the head, neck and forelegs out as one unit, the hind legs and tail as a second unit and preserve these in liquid. The stomach and reproductive organs should also be preserved in liquid. Carefully clean out and dry the shell.

3. Crocodilians- Measure and skin the specimen as for snakes, except that the tails should be skinned as well. Feet may be left attached (inject with fixative), instead of skinned out. Rub the skin with borax or arsenical soap and dry.

SHIPPING

Once they have been properly fixed, most herpetological specimens may conveniently be transported by wrapping them loosely in cheesecloth or white paper towel which has been liberally moistened with alcohol (70% ethanol or 40% isopropanol), or the fixative, then sealing them in two plastic bags (one within the other, individually closed by twisting the end and knotting or securing with a rubber band). Several small specimens may be wrapped in a single length of cheesecloth by laying the cloth out flat, spacing the specimens down the length of it, folding the sides over the animals and rolling the cloth loosely, like a rug.

Thus packed, the specimens occupy minimum space and weight, important factors when they must be transported any distance or mailed. Specimens will remain in

good condition for several weeks, so long as the bags are well sealed to retard leakage and evaporation. Cotton in sheet form may be substituted for cheesecloth, but is bulkier and may adhere to the scales of some rough-scaled species of lizards, even when it is moist.

Specimens being shipped (parcel post is a convenient means) should be carefully packaged and clearly marked "PRESERVED SCIENTIFIC SPECIMENS." Packages often are subject to much "wear and tear," so, effort in preparation pays off! New paint cans of varying sizes make leakproof, sturdy mailing containers. Plastic bags containing specimens may be simply placed in the can and extra space filled with wadded rags or paper. Bags with heavy specimens should never be placed on top of lighter ones. Three address labels should be typed or written with permanent ink. Place one of these in the can with the specimens, tape a second to the side of the can. Affix the third label to the paper used to wrap the parcel.

Paint cans are not too costly, and a source of supply can generally be found by consulting local paint stores. Watching auction notices sometimes turns up a paint store that is going out of business and may have cans. Remove handles from cans before use. In lieu of cans, specimens may be packed in any durable container. Check postal regulations for size and weight restrictions before packing extremely heavy or unwieldy parcels. Specimens such as large turtle shells or the skins of large crocodilians may have to be sent via freight, and again, secure packing is a must. Shipment of all crocodilian specimens is subject to stringent regulation, as many of these species are endangered animals. Collectors planning to take these should carefully check customs regulations for import restrictions as well as checking capture laws in countries where the animals occur. Proper arrangements can often be made through the institution where one proposes to deposit the specimens.

Generally, specimens should never be sent in glass containers. Obvious exceptions to this are amphibian eggs (and sometimes, larvae) and very fragile specimens. These should be placed in the smallest containers needed to hold the specimens plus fluid to maintain them; fluid should fill the containers, which must be heavily padded with cardboard or cotton. If rigid plastic tubing of sufficient diameter is available, break resistant containers may be fashioned from it by cutting an appropriate length, stoppering one end, enclosing specimens and fluid, then sealing the other end. The tube may be wrapped lengthwise with

wire to secure the stoppers. Plastic vials are available from some biological supply houses; larger drugstores may also furnish the names of suppliers of these. Be sure to only use vials which can be securely closed (screw-on or snap-on lid). Again, pay special attention to wrapping such containers.

INTERNATIONAL SHIPMENTS

Collectors should be aware of proper methods for shipping specimens internationally. Donation of all or part of a collection to institutions outside one's own country serves to:

1. make synoptic herpetofaunal collections of different areas available to as many researchers as possible,

2. prevent the loss (through war, neglect, earthquakes and other damage) of valuable collections deposited entirely in a single institution, and

3. place the herpetologist in contact with colleagues in foreign institutions; this frequently leads to a most beneficial exchange of ideas and data, thus advancing herpetology as a field of study.

The private hoarding of specimens by any person is a waste of valuable biological data, and can lead to overcollecting (i.e.--researchers may gather specimens from areas already represented, though inaccessible, in private collections). It is with the above points in mind, and the hope that more collectors will decide to enter into donation, exchange or loan relationships with foreign institutions, that the following guidelines are presented.

The methods of packing described in the preceding section are adequate for international shipment. Generally, mail is the most convenient means of sending packages which are not too heavy or bulky. Parcels sent via surface ("ordinary") mail should have extra preservative added to the specimen bags, as they may take as long as 4 months to reach their destination. Persons

mailing specimens internationally should check local mail regulations on parcel size, weight and any special packing provisions. The shipper may also be required to affix various postal and customs "declaration tags" to parcels. These tags vary with parcel destination and are generally provided by the postal service.

Very large or heavy packages will have to be sent via freight (air, train, ship). The sender will be required to complete a "waybill" (available from the carrier) listing, among other things, the nature and value of the contents. To avoid excess charges, package and waybill should be marked "No Commercial Value." Postal services in all countries have the legal right to inspect all packages. Intensive efforts to curtail the traffic of narcotics and other restricted drugs has led to the extensive exercising of this right, and the fact that several persons have attempted to smuggle drugs within specimen containers has not aided the situation. Inspectors often open plastic bags of specimens, and may be unaware of the need to reseal them. This causes loss of fluid and dehydration and probable loss of the specimens. It is therefore advisable to include two copies of the following statement with the parcel (one pasted on the outside and one sealed within):

INSPECTION OFFICER: This package contains dead, preserved amphibians and/or reptiles packed in plastic bags. As the specimens have great scientific value and will be ruined if not kept moist in their preservative, it is imperative that the bags be tightly resealed after inspection to avoid evaporation or leakage of preservative. Thank you.

INSPECTOR POSTAL: Este paquete contiene ejemplares de anfibios y/o reptiles muertos, preservados y empacados en bolsas plasticas. Puesto que los ejemplares son de valor cientifico y se arruinan si no permanecen en su liquido preservativo, se suplica que, despues de abrir las bolsas para inspeccionarlas, las cierre hermeticamente para evitar que el liquido se evapore o se derrame. Gracias.

AS AUTORIDADES ALSANDEGARIAS: Este volume contem anfibios e repteis mortos, conservados em sacos plasticos. Como o conteudo tem valor cientifico e se estragara se nao for mantido humido no preservativo, pedese que apos abrir os sacos para inspecao os mesmos sejam firmemente fechados para evitar evaporacao ou derramamento do liquido. Obrigado.

Biologists should also be aware that the international shipment of specimens (alive or preserved) is being ever more closely regulated for conservation reasons. Shipments of preserved animals sent to the USA must be accompanied by a list bearing the number and scientific name of all specimens included. The importer (in the USA) must obtain a special permit from the Bureau of Sport Fisheries and Wildlife (Dept. of the Interior) in order to receive foreign shipments of preserved or live specimens.

Live shipments are additionally regulated by the Dept. of Agriculture and the Public Health Service. In all cases, endangered species are covered by regulations separate from species not currently considered endangered. You are urged to carefully investigate all legal aspects of international shipment before preparing to send animals.

STORAGE AND LABELLING

This section is not intended to be a complete guide to curatorial technique. Rather, it is meant to serve as a set of capsule directions for those wishing to start a preserved herpetological collection. A detailed discussion of curatorial technique may be found in Slevin (1927).

Preserved collections are best maintained in alcohol. Suitable alcohol generally costs about the same (per gallon) as formaldehyde, and alcohol-stored specimens are far easier to work with. Formaldehyde also tends to corrode metal lids and containers. Most collectors will be deterred from using ethanol by the high tax imposed upon its sales. Isopropanol is far cheaper, and is entirely satisfactory for storage of specimens. Methanol should never be used. Concentration of 50% is suitable for reptiles, while 40% is better for amphibians. Both ethanol and isopropanol are generally sold at 95% concentration; 526 ml of this plus 474 ml of water make one liter of 50% concentration (421 ml alcohol + 479 ml water for 40%). Specimens being transferred from formalin or FAA fixation to alcohol must first be soaked in water for 48 hours. Failure to soak the specimen often results in its being severely dehydrated by the alcohol. Properly fixed specimens will not be harmed by this method. If material is desired for use in histological work, selected pieces of tissue should remain in 30% alcohol

for 24 hours, then 24 hours of 50% alcohol before going to final storage (omit the water soak). Do not pack specimens tightly in the jar. Snakes fixed in the position illustrated earlier will readily coil in jars for storage.

Each specimen retained in the collection should be assigned a catalog number (in addition to the aforementioned "field number"). Amphibian eggs and larvae and reptile eggs may be cataloged with a single tag designating one clutch or lot. This number should be entered in a permanent catalog (using waterproof ink), along with the species name, date of capture/preservation, sex, locality, ecological notes and name of collector. Tags may be tied in the same region as the field tag. Collector's field number should also be entered. As a cross-reference, it is useful to maintain a card file (by taxonomic family) in which a single card is used for each species. On this card may be entered numbers from the catalog that apply to these species.

It is convenient to place a label bearing species name, catalog numbers and locality data with each container. These should be written in permanent ink on heavy, durable paper. That produced by Byron-Weston Mills under the name "Linen Record Ledger", 100% cotton and linen fiber, 36 lb. and Dennison Paper Company's product "Resistall Index Bristol", 100% rag, 110 lb. wt. are both excellent and are available through printing shops. The label may either be placed within transparent containers, or attached to the outside of opaque ones with masking tape. If moderate cost can be withstood, external labels can be placed within tie-on, plastic label-holders. A typical museum label is shown in Figure 2.

Specimen jars should be stored in cool places to help retard evaporation of preservative, and should never be exposed to sunlight, as specimen colors are rapidly faded by such exposure. Placing a piece of Parafilm sheet (available from Carolina Biological Supply House, Burlington, North Carolina) over jar mouths before screwing on the cap will also reduce evaporation. Containers should be checked periodically and fluid level maintained. Well preserved and cared for collections make valuable teaching and research tools.

COLOR PRESERVATION

While preserving the morphological state of herpetological specimens has never presented any severe hurdles to collectors, preservation of color is quite another matter. All currently used, popular preserving fluids are alcoholic and/or acidic to some degree. Therefore, it usually is not too long before most pigments are dissolved by such fluids and extracted from the specimens. Amphibians seem particularly vulnerable in this regard, though the effect on reptiles is noticeable.

Previously, the only acceptable method of retaining amphibian skin color was that described in Cook (1965). Basically, this consists of skinning the specimen, confining all cuts to the ventral surfaces of body and limbs. The skin is then floated flesh-side up in a pan of water and remaining particles of tissue are removed. The skin next is floated flesh-side down and spread out in a second pan. A wet piece of cardboard may then be brought up beneath the skin, which is rubbed lightly to flatten it and remove trapped air. The cardboard-skin preparation may be dried on blotting paper until moist, then placed between layers of blotting paper and thoroughly dried with heavy weights (such as books) on top of it; it may also be placed in a plant press. Reptiles may be similarily prepared. In all cases, the carcass should be preserved in fluid and tagged with the same number as the skin. Skins thus prepared should be stored in the dark and not exposed to prolonged light.

The above technique, while useful, is tedious. Windsor (1971) has described a technique for using 50% saturated, aqueous ammonium sulfate solution as a preservative of frogs. As the compound is an aqueous, neutral salt, no pigment was dissolved and natural color was still evident in the specimens 6 months after preparation. Total fixing time should be at least 36 hours.

Specimens may be stored in buffered formalin (10%) or isopropanol (40%) to which liquid Ionol-40 R has been added (White and Peters, 1969). Storage should be in dark places which are not subjected to heat much above 70^{0}F. The formalin/Ionol method has been successfully used with herpetological material by Mr. Woodrow Barber, Biology Department, University of Kentucky at Morehead, and by Dr. George Iannarone, Chicago Academy of Sciences (personal communication).

Powdered Ionol should not be used, as it is difficult to prepare a stable solution of it in preservative. Ionol is sold by the Shell Oil Company (Chemicals Division).

While the two chemical methods discussed above have not been widely used with herpetological material, their success on a limited scale coupled with the value of accurate color preservation suggests that they should be more thoroughly investigated.

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